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A PRESOMITE HUMAN EMBRYO (SHAW) WITH PRIMITIVE STREAK AND CHORDA CANAL, WITH SPECIAL REFERENCE TO THE DEVELOPMENT OF THE VASCULAR SYSTEM

By R. J. GLADSTONE and W. J. HAMILTON

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INTRODUCTION

The embryo was presented to the Anatomy Department of the Medical College of St Bartholomew's Hospital, London, by Dr Wilfred Shaw. In appreciation of the gift we have associated the name of Dr Shaw with this embryo.

The embryo shows many features of interest in the development of the angioblastic tissue and blood cells. These features have been described at some length, since they represent an intermediate stage of development which throws considerable light on the relations between angiogenesis and blood formation. In the chorionic mesoderm, including that of the villi, the outstanding feature is the presence in the mesenchyme of angioblastic strands of nucleated protoplasm which, by vacuolation, are becoming transformed into sinuses lined by endothelium and capillary vessels, of which the majority appear to be empty and contain at this stage of development no cellular elements. In the yolk sac haemopoiesis dominates and angiogenesis appears to be secondary. This intermediate stage bridges over a gap in the history of our knowledge of the development of the vascular system in the human subject and confirms the opinion of Streeter (1920), Ingalls (1921), and other authors with regard to the early and advanced stages of angiogenesis in the chorionic mesoderm as compared with blood formation, while in the yolk sac the blood islands often appear before the endothelial walls which are subsequently developed around them.

A short description of this embryo was published by Dr Shaw in 1932, and the following account of the clinical history of the case is based on his original article.

The embryo was obtained at a subtotal hysterectomy performed by Dr Shaw, on a woman aged 32, for the relief of a right cornual uterine myoma. The uterus was opened immediately after removal. A small prominence, about 1 cm. in diameter, was observed on the posterior wall. This was excised and fixed in Carnoy-sublimate solution for 1 hr. and then passed through alcohol, xylol, benzene and wax in the usual way. Dr Shaw cut a complete series of sections of the whole block of tissue in which the embryo was embedded, at 10 μ thickness. The histological condition of the specimen proved to be exceptionally good. and the embryo was neither diseased nor macerated.

The woman had had two previous pregnancies. One child had been born at term in 1922, and this was followed by menorrhagia, but the sequence of the menstrual cycle was unaltered; in 1926 the patient had a miscarriage during the fourth month. On 17 February 1930 she came to St Bartholomew's Hospital complaining of an abdominal tumour and abdominal pain. She had last menstruated between 12 and 17 January 1930. Her menstrual period was one of 28 days; the next period should accordingly have started on 9 February, 8 days before she came to the hospital.

Rising from the pelvis was an abdominal tumour 4 by 3 in. in diameter, which on bimanual examination was located in the right cornu of the uterus. The patient was admitted into the hospital, and the operation of subtotal hysterectomy was performed 2 days later, on 19 February. The left ovary was removed and found to contain a corpus luteum.

In discussing the data bearing upon insemination, ovulation and fertilization, Dr Shaw ascertained that the probable date of insemination was 24 January. A discrepancy in the history occurred between the patient's assertion that the February period was due on the 12th and the calculated period 9 February, when the period should have appeared in conformity with her normal period of 28 days. The dating of the specimen, calculated from 19 February, the date on which the operation was performed, is as shown in Table 1.

Table 1

Accredited dates of menstruation, insemination, ovulation, and assumed age of embryo	Number of days preceding the operation date, 19 Feb.
From onset of last menstruation (12 Jan.)	38
From assigned date of insemination (24 Jan.)	26
From assumed period of ovulation (25–28 Jan.)	25 to 22
From calculated date of expected menstruation (9 Feb.)	10
From onset of next menstruation expected by patient (12 Feb.)	7
Assumed 'fertilization age' of embryo	23 to 20

Assuming that fertilization took place within 48 hr. of ovulation, the age of the embryo, according to the data given above, would be from 20 to 23 days. Presomite embryos belonging to group E of Bryce's classification (1924) or to groups V or VI of Hertig's classification (1935), with which this embryo corresponds, namely, embryos with a completed notochordal process (head process of many authors) and chordal canal (archenteric canal of many authors), but before the development of neural folds, are usually thought to be younger than this, e.g. the Heuser (1932) presomite embryo (Carnegie, no. 5960) has an estimated 'ovulation age' of 18–19 days, as contrasted with that of the present Shaw embryo of 20–23 days.

In discussing the relation of ovulation to fertilization, Dr Shaw, after stating that the general belief is that ovulation is limited to the intermenstrual phase of the cycle, mentions that, in his opinion, 'ovulation is limited to

between the 13th and 16th days of the menstrual cycle, the first day of the period of bleeding being taken as the first day of the cycle', and that, 'in the human subject it is uninfluenced by such external factors as coitus' (see also Shaw, 1925).

Further, he stated that, in his view, 'since it is obvious that fertilization cannot precede ovulation, the union of spermatozoon and ovum must be regarded as being restricted to the latter half of the menstrual cycle, but this view does not imply that insemination before the date of ovulation must be sterile, there being much statistical and clinical evidence that coitus may be fertile during the first half of the menstrual cycle (Siegel, 1916).

Allen *et al.* (1930), who have recovered living ova in washings from the uterine tube, and who have studied the young corpora lutea, believe that ovulation takes place on or about the 14th day of the cycle. The recovery of ova is the most convincing method of demonstrating that ovulation has occurred.

The work of Knaus (1934), Venning & Browne (1937), and others confirms the view that ovulation occurs around the 14th day of the cycle.

It is possible that this difference in the degree of development of the Shaw embryo, as compared with others, may be explained by retardation of development due to the existence of a myoma of the uterus, although, judging from the sound condition of its tissues, the embryo itself does not seem to have been diseased. Other possibilities are: (*a*) ovulation had occurred at a later date in the cycle than between 25 and 28 January (see Flew, 1941), (*b*) a delay in fertilization had taken place—in other words, there was an increase in the period between ovulation and fertilization, and (*c*) the date of fertile insemination was not 24 January, as the patient admitted that coitus interruptus had been frequently practised during January and February.

GENERAL DESCRIPTION AND DIMENSIONS OF THE SPECIMEN

The chorionic vesicle which is embedded in the compact layer of the decidua is almost completely covered by a decidua capsularis. It belongs to group E of Bryce's classification (1924)—in which a primitive streak and cloacal membrane are differentiated and a complete chorda canal has been formed; in the case of the Shaw embryo the canal appears to be a blind diverticulum. The intrachorionic rudiment (amnio-ectodermic and yolk-sac vesicles) is surrounded by the chorion with its villi and has the embryonic plate included within the opposed walls of the two vesicles of which the yolk sac is the larger. The intrachorionic rudiment is connected with the internal aspect of the wall of the chorionic vesicle by a connecting stalk which consists of two parts, a basal 'amnio-embryonal' segment, and a proximal 'umbilical' segment containing an allanto-enteric diverticulum (Text-fig. 3).

The principal measurements of the specimen are given in Table 2.

Some of these measurements have been made directly from the sections mounted on the slides, others have been estimated indirectly by counting the number of sections (10 μ thick) in which a particular part appears in the series. Owing to shrinkage and the obliquity of the sections to the longitudinal axis of the embryonic plate the measurements must be regarded as approximate (see Text-fig. 1). In the case of an elliptical disc obliquity of the sections relative to the axis will cause a *diminution* in the estimated length of the longitudinal axis, and an *increase* in the width, which will be proportional to the degree of deviation of the plane of the sections from that of the embryonic disc; the estimation will also be influenced by the difference between the length of the longitudinal or axial plane of the ellipse and the width of the ellipse.

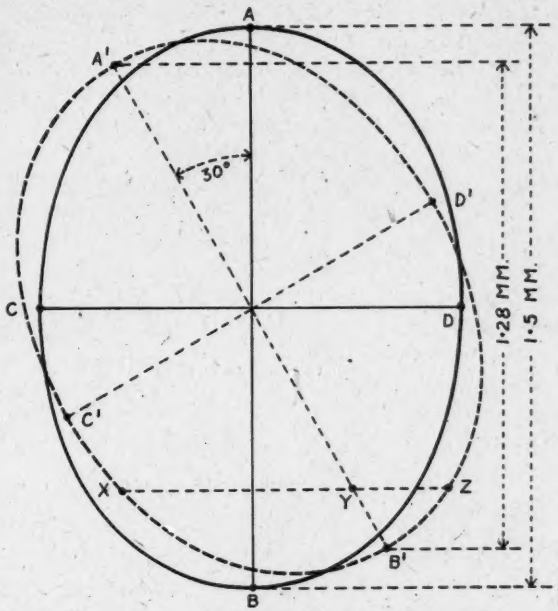
Table 2

Approximate measurements	mm.
Maximum horizontal diameter of infiltrated zone around the implantation cavity	15
Depth of infiltrated zone, including the closing plate	5
Chorionic vesicle: maximum external diameter, including villi	11
Chorionic vesicle: vertical external diameter	4·04
Chorionic vesicle: maximum internal diameter	8
Chorionic vesicle: vertical internal diameter	3
Embryonic rudiment	1·66 × 1·34
Length of embryonic disc	1·05
Body stalk	0·61
Amnion	1·35 × 1·34 × 0·32
Yolk sac	1·21 × 1·35 × 0·40
Allanto-enteric diverticulum	0·35
Cloacal membrane	0·09
Definitive	0·05
Caudal	0·04
Primitive streak	0·43
Preblastoporic segment of disc	0·62
Hensen's knot	0·04
Notochordal process	0·17

Another well-known circumstance which renders the measurement of embryos difficult is the development of the normal sagittal and transverse curvatures of the embryo, and the occasional presence of abnormal twists and tilts. Towards the end of the presomite phase of development the ventral flexure of the caudal end of the embryo has a marked effect upon the estimate of the length of the primitive streak, and at a later period when the cephalic flexure is being formed estimation of the length of the embryo by the simple method of enumerating the number of sections is still more profoundly affected. In the specimen under consideration, the ventral bend of the caudal segment of the primitive streak renders any measurement of its length, except upon a model, extremely difficult, since a very considerable extent of its total length is included in two or three sections. This is due to the plane of the sections which pass through the terminal ventrally flexed part of the primitive groove

being somewhat in the longitudinal axis of the groove, instead of transversely as in its anterior part.

In many specimens, owing to twists around the longitudinal axis and bends in a lateral direction, measurements based on linear reconstructions are also liable to be fallacious. An indication of the amount of error that may occur by simple enumeration of sections, even in a perfectly ellipsoidal or approximately



Text-fig. 1. *ACBD* is an ellipse representing the outline of an embryonic disc in which it is assumed that the primitive streak and groove occupy the lower half of the major axis of the ellipse, and that the serial sections have been cut at right angles to the major axis. *A'C'B'D'* represents an embryonic disc of corresponding size and form the sections of which have been cut at an angle of 30° with the major axis of the ellipse. The maximum length of the disc, if calculated from the number and thickness of the sections which include the major axis, would be shortened by 0·22 mm. and the primitive groove would also be proportionately shortened by 0·11 mm. *Y* in the interrupted line *XYZ* represents the position in which the primitive groove would appear in relation to the edges *X*, *Z* of the embryonic disc in a section cut in the position which is indicated in the figure.

circular embryonic disc, will be gained by a reference to Text-fig. 1, and also the advantage which is gained by an attempt to make a reconstruction model will be appreciated, even though an orientating or basal line may be absent. In the present case, the absence of a base-line was overcome by making use of a portion of the wall of the chorionic vesicle in the region of the embryonic rudiment.

The chorionic vesicle

The chorionic vesicle is flattened in such a way that its major axes lie parallel to the membrane lining the posterior surface of the uterine cavity in which the vesicle was embedded. The external surface of the sac is covered throughout its whole extent by villi, and these are more differentiated and larger at the circumference than at the embryonic pole (future placental site) or at the abembryonic pole (next the lumen of the uterus).

The epithelial layer of its wall shows a distinct subdivision into cytotrophoblast and syncytiotrophoblast (Pl. 3, figs. 7, 8). The extent and relations of the latter to the uterine mucosa will be described in a subsequent publication. The cytotrophoblast has the usual characters of this layer in embryos at this stage of development. Mitotic figures are occasionally visible in it, and connexions with the underlying mesenchyme and angioblastic strands appear to be present; these are discussed in the description of angiogenesis and haemopoiesis.

The chorionic mesoderm consists of a thin stratum of mesenchymal tissue, composed of a network of laminated fibres (Pl. 3, figs. 7, 8), with nuclei at the nodes of the reticulum and enclosing intercellular tissue spaces. Within the mesenchymal tissue there are also strands of angioblastic tissue, many of which contain vacuoles, which by fusion give rise to intracellular spaces; the latter eventually, by further fusion and extension, become the lumina of capillary vessels and sinuses.

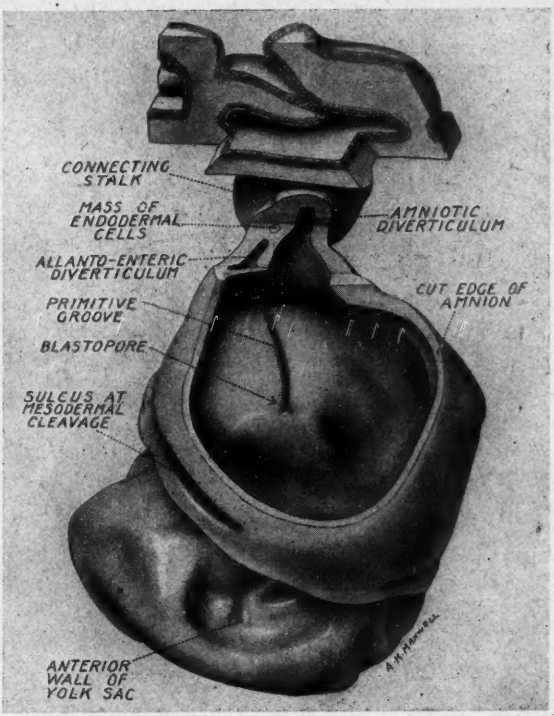
The spaces are, therefore, of two types: (1) intercellular and (2) intracellular. The intercellular spaces are irregular in form and represent the meshes of the network (Pl. 3, figs. 7, 8). They contain occasional free cells of a lymphoid or phagocytic type. The intracellular spaces at this stage of development usually contain no formed elements except near the attachment of the connecting stalk (Pl. 4, fig. 9). During life they are probably filled by embryonic plasma, secreted by the endothelium. The mesenchymal tissue, on its internal aspect, is limited by a flattened mesothelial stratum which is in relation with the magma reticulare. The mesothelial stratum, however, is in some places incomplete, and the mesenchymal tissue becomes continuous with delicate syncytial strands of the magma reticulare. The latter contains, in addition to the protoplasmic strands, groups of cells of both mesodermal and epithelial types. There are also isolated cells showing various stages of degeneration, and some free pycnotic nuclei. The epithelial cells appear to be of both entodermal and ectodermal origin. Some of the former are found in close relation with the yolk sac and possibly may have originated from a yolk-sac diverticulum or stalk, while the ectodermal cells are found in the vicinity of the body stalk and amnion.

The embryonic disc

The embryonic disc is 1·05 mm. in length and 1·34 mm. in breadth. Including the body stalk the length of the whole embryonic rudiment is 1·66 mm. The primitive streak part of the disc is 0·43 mm. in length and the preblastoporic segment is 0·62 mm.

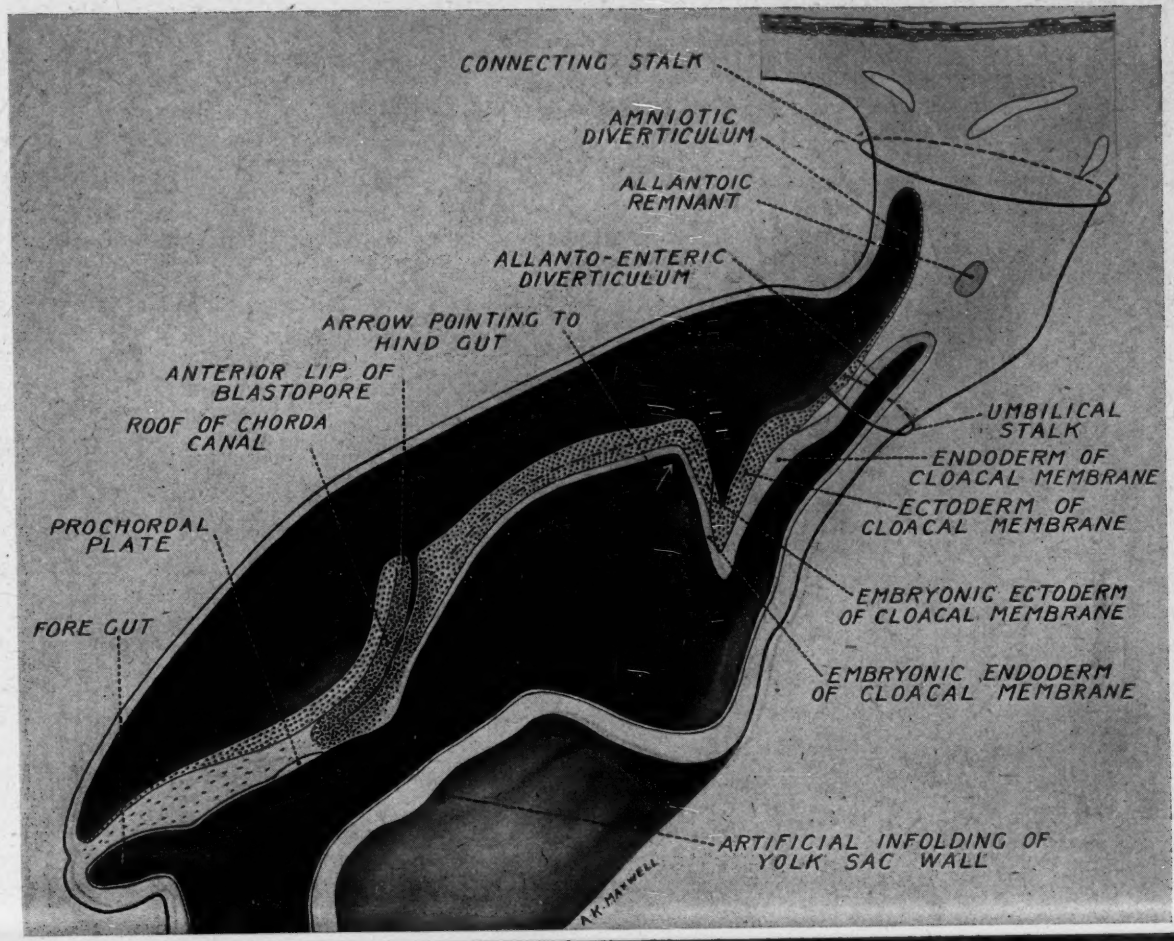
The disc is somewhat oval in outline (Text-fig. 2). In the coronal plane it is curved with a slight dorsal convexity, but at the margin of the disc the convexity is replaced by a slight concavity (Text-fig. 4).

In the median axis of the disc, in its posterior part, there is a distinct primitive groove which overlies the primitive streak (Text-fig. 4). A blastopore is situated at the front part of the primitive groove, and in front of the blastopore there is an elevation produced by its anterior lip. The disc in front of this dips away in a moderately steep slope to the anterior edge (Text-fig. 3).



Text-fig. 2. Wax plate reconstruction of the embryo made at a magnification of 100 diameters and reduced to a magnification of 50 in the reproduction. Viewed from above and in front.

The ectoderm is composed of a pseudo-stratified layer of tall cells (Pl. 2, fig. 4 and Pl. 3, fig. 6) which become flattened at the edge of the disc, where they are continuous with the ectodermal cells of the amnion (Text-figs. 4–6); they have a distinct basement membrane except in the regions of Hensen's knot and the primitive streak. The nuclei of these cells are round or oval and contain a distinct nucleolus. Numerous mitotic figures are present in the ectodermal cells of the primitive groove and near its margins. These


CONNECTING STALK
AMNIOTIC DIVERTICULUM
ALLANTOIC REMNANT
ALLANTO-ENTERIC DIVERTICULUM
ARROW POINTING TO HIND GUT
ANTERIOR LIP OF BLASTOPORE
ROOF OF CHORDA CANAL
PROCHORDAL PLATE
FORE GUT
UMBILICAL STALK
ENDODERM OF CLOACAL MEMBRANE
ECTODERM OF CLOACAL MEMBRANE
EMBRYONIC ECTODERM OF CLOACAL MEMBRANE
EMBRYONIC ENDODERM OF CLOACAL MEMBRANE
ARTIFICIAL INFOLDING OF YOLK SAC WALL
A.K. MAXWELL

proliferating ectodermal cells give rise, on each side of the primitive streak, to intra-embryonic mesoderm. Towards the anterior part of the disc the number of nuclear layers in the ectodermal cells becomes fewer—compare Text-figs. 7–9 with Text-fig. 4 and Pl. 3, fig. 6.

In the region of the disc in front of Hensen's knot the surface of the ectodermal epithelium is irregular and some of the cells appear to be undergoing degenerative changes (Pl. 2, fig. 4). Whether this is the result of tension in a rapidly growing area or the result of fixation cannot be determined.

At the posterior part of the primitive streak there is great proliferation of the cells of the ectoderm. Some of these cells contribute to the formation of the mesoderm of the connecting stalk and pass around the side of the cloacal membrane into the stalk (Pl. 3, fig. 5).

There is no evidence yet of the limitation of the medullary plate, nor is there as yet any sign of the formation of the medullary groove.

Cloacal membrane

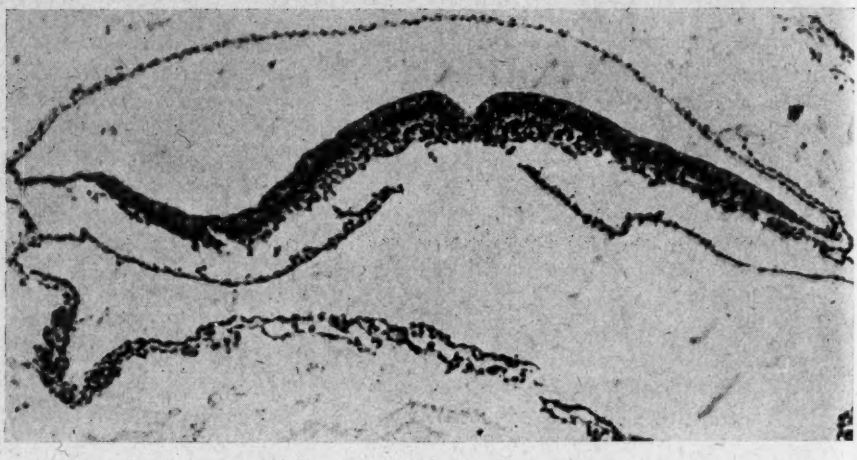
This area is represented by the fusion of ectoderm and entoderm behind the primitive streak. It involves the posterior part of the embryonic disc and the proximal portion of the allanto-enteric diverticulum. The extent of these two parts is seen in Table 2 and is shown schematically in the graphic reconstruction (Text-fig. 3). The ectodermal cells of the embryonic disc and amniotic wall which form the ectodermal part are cuboidal or columnar and have rounded nuclei. The entodermal cells which come into contact with the ectoderm form a somewhat conical mass; their nuclei are oval in shape (Pl. 3, fig. 5). The mesodermal cells which border the sides of the membrane are separated from the entodermal cells.

Primitive streak and mesoderm

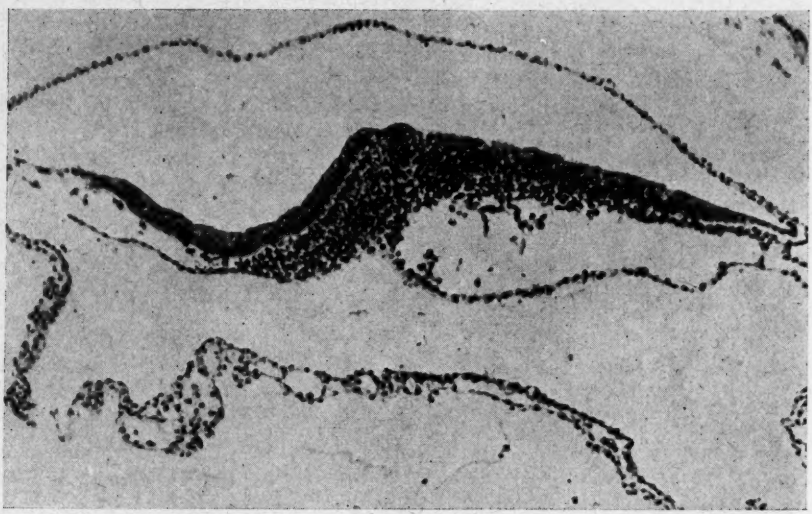
The extent of the primitive streak is given in Table 2 (see also Text-fig. 3). The mesoderm cells pass laterally from the sides of the streak as a layer which divides into two at the edge of the disc (Text-fig. 4). The amount of mesodermal tissue is much greater at the posterior half of the disc where it forms a compact mass of cells, than in the anterior half where it is only represented by scattered cells and does not form a continuous sheet (compare Text-fig. 4 with Text-figs. 8, 9). Whether any mesoderm arises from the side of the chordal process could not be determined owing to the obliquity of the sections. The entoderm forms a distinct layer below the mesoderm except in the middle line below the primitive streak where it is intimately related to the mesodermal tissue.

Hensen's knot and chorda canal

There is great proliferation of the ectoderm in the region of Hensen's knot which is raised above the general level of the ectoderm (Text-fig. 3 and Pl. 2, fig. 4). The posterior edge of the knot is sharply delimited from the anterior extremity of the primitive streak. A blastopore leads into a chorda canal; at



Text-fig. 4. General view of the embryonic disc at the anterior part of the primitive streak and groove. Blood islands in an early stage of development are seen to the left and right; in the centre, endothelial-lined spaces, some of which contain free blood corpuscles mostly of primitive erythroblast type. Section no. 57, the sections having been numbered from the anterior end of the embryonic disc. × 100.



Text-fig. 5. General view of the embryonic disc in the region of Hensen's knot; the section is to one side of the blastopore (compare with Pl. 2, fig. 4). Vascular spaces containing a few free developing blood corpuscles are visible in the lower part of the section; they are lined on their distal aspect and sides by endothelioid cells with rounded or lens-like nuclei; on the central side, however, the mesothelial layer is often defective and the wall of the space is formed by entoderm. Section no. 47. × 100.

its commencement the latter is a cavity, but farther forward it is only a potential space, and its anterior end appears to be solid (Text-figs. 3, 5). The cells in the roof of the canal are tall, columnar, have a clear cytoplasm, and their boundaries are mostly well defined. The nuclei of the cells are deeply stained and are at, or near, the base of the cells. The cells of the floor of the canal are intimately connected to the underlying entoderm, which in this region does not appear as a distinct layer (Pl. 2, fig. 4). The chorda canal, as far as can be determined, does not communicate with the yolk sac. Owing to the obliquity of the sections it is not possible to determine accurately the anterior end of the notochordal process, nor is it possible to state whether mesoderm arises from the sides of the notochordal process.

Prochordal plate

In the present specimen, the prochordal plate is irregular in shape, two horns projecting backwards from its posterior edge on each side of the anterior end of the notochordal process. The plate is not sharply delimited from the surrounding entoderm and it is composed, for the most part, of cuboidal cells in some of which are chromophilic granules (Pl. 3, fig. 6). Dorsal to the plate, in some sections, there is some cell detritus and many chromophilic granules. The plate is separated from the ectoderm, and only a few mesodermal cells are interposed between it and the ectoderm.

Connecting stalk

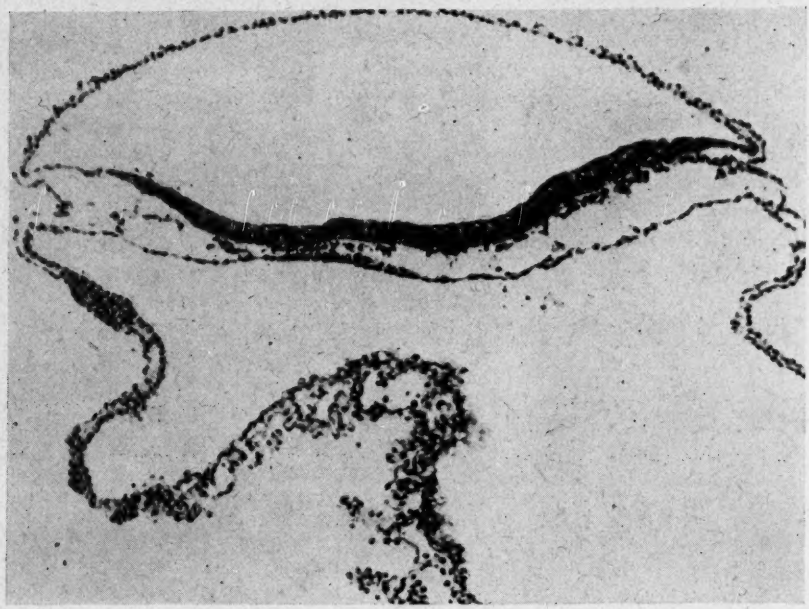
Following the description of Florian (1930), the connecting stalk may be divided into two parts, the amnio-embryonal stalk and an umbilical, or body, stalk. The latter makes an angle of 30° with the embryonic disc. The whole connecting stalk is composed of a mass of mesenchymal cells, projecting into which are the allanto-enteric diverticulum and a diverticulum from the dorso-caudal part of the amnion (? amniotic duct) (Text-figs. 2, 3). The mesenchymal tissue at the base of the stalk (Pl. 4, fig. 9) consists of a wide-meshed reticulum containing intercellular spaces and enclosing angioblastic strands and developing capillary vessels; in the region of the allanto-enteric diverticulum (Pl. 2, fig. 3) the cells are rounded and have an oval or spherical nucleus. The cells are thus more compactly arranged around the allanto-enteric diverticulum than in the more basal part of the stalk. The allanto-enteric diverticulum arises from the right side of the yolk sac. This is probably due to the fact that the embryo has been twisted upon the stalk as is so commonly found in young embryos. The umbilical stalk is bounded in front by the amniotic ectoderm. There is some irregularity in that part of the amniotic wall which comes into contact with the mesoderm of the stalk. Some of the amniotic ectodermal cells show degenerative changes, and some have become freed into the amnion. The appearance is similar to that described by Florian and suggests the enlargement of the posterior part of the amniotic cavity at the expense of the

stalk tissue. Chromatic particles are present in some of the ectodermal, mesodermal, and entodermal cells in the anterior part of the stalk (Pl. 2, fig. 3). Well-formed blood vessels and blood islands are present in the stalk; their appearance is described under angiogenesis and blood formation.

Yolk sac and allanto-enteric diverticulum

The measurements of the yolk sac are given in Table 2.

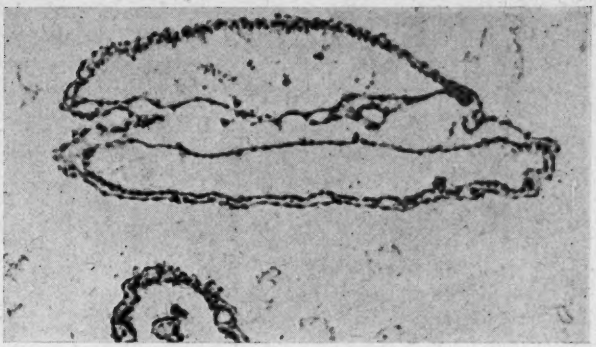
There is an infolding of the abembryonic part of the sac (Text-figs. 3, 6), and in our opinion this is an artefact. Further, the sac does not lie symmetrically below the embryonic disc but more to the right side of it, possibly as the result of the twisting of the upper part of the embryo (Text-fig. 2).



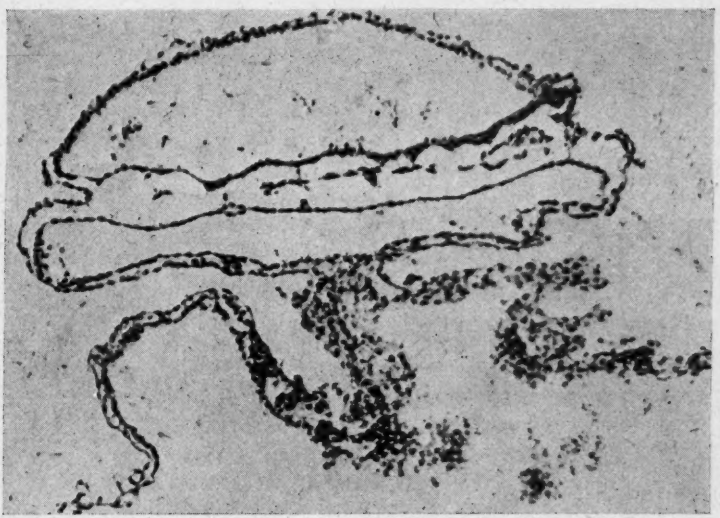
Text-fig. 6. General view of the embryonic disc showing the relations of the thickened entodermal cells forming the prochordal plate. Typical blood islands are seen in the wall of the yolk sac on the left side of the photograph. Vascular spaces are visible in the lower part of the figure. They contain a few free corpuscles, or are quite empty, vasculogenesis being more advanced in the abembryonic region than at the circumference. Section no. 31. × 100.

At the anterior end of the embryo there is an infolding from the front and, to a lesser extent, at the sides of the wall of the yolk sac to form what we interpret as the foregut (Text-figs. 7–9).

At the posterior end of the embryo there is a folding of the posterior part of the primitive streak and embryonic part of the cloacal membrane to form the beginning of the hindgut (Text-fig. 3). The allanto-enteric diverticulum



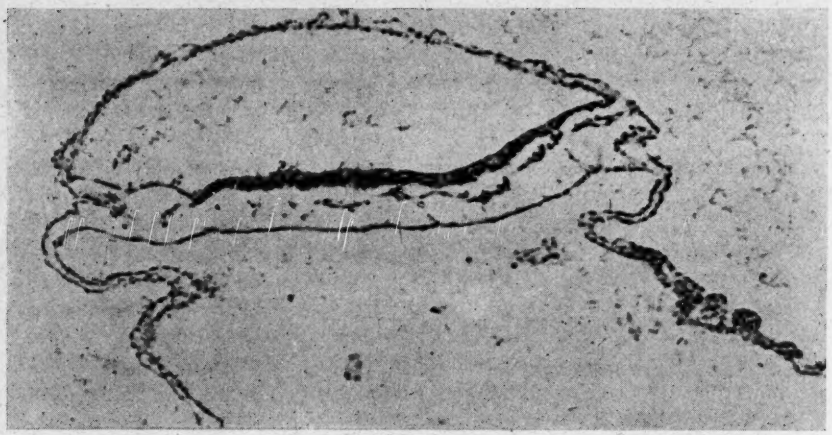
Text-fig. 7. Section through the anterior part of the embryonic rudiment showing amnion, embryonic plate, and wall of the yolk sac. The wide cavity beneath the embryonic plate is believed to be the foregut. The mesoblastic tissue between the embryonic ectoderm and the entoderm is here scanty and in the section appears incomplete, this section lying in the presumptive region of the buccopharyngeal membrane. The mesenchymal cleft to the left in the figure, and the enclosed space in the corresponding situation on the right, correspond in position to the future pericardial cavity. A part of the anterior wall of the yolk sac is seen in the lower part of the photograph. Empty spaces lined by endothelial cells are present in the wall of the yolk sac and embryonic plate. Section no. 6. ×100.



Text-fig. 8. The section passes through the anterior boundary of the anterior intestinal portal and shows the junction of the lateral folds of the yolk sac wall. Blood islands and empty endothelial-lined spaces are present in the wall of the yolk sac, and in the floor and roof of the foregut. Section no. 10. ×100.

arises below the level of the hindgut, passes to the right and upwards into the body stalk, and its tip is in close relationship with a detached mass of entodermal cells.

The entodermal layer of the yolk sac is formed of cells the shape and size of which differ considerably in different parts of the sac. The entodermal cells below the embryonic disc are flattened, except in the prochordal plate region (Text-fig. 6 and Pl. 3, fig. 6) where they are cuboidal, and in the floor of the foregut where they become rounded (Text-fig. 7). In the region of the cloacal membrane and allanto-enteric diverticulum they are again cuboidal. In the abembryonal pole of the yolk sac, where the vascular spaces are well developed,



Text-fig. 9. A section just behind the anterior intestinal portal. The lateral infoldings of the yolk sac wall, and the marginal clefts in the mesenchyme of the embryonic plate in the region of the pericardium, are visible. The medullary plate which in Text-figs. 7 and 8 consists of a single layer of flattened ectodermal cells is now considerably thicker and two or three layers of nuclei are visible. Section no. 12. × 100.

some of the entodermal cells are columnar and swollen, others contain large vacuoles in their free surfaces, while still others show a ragged edge or are rounded, the vacuoles apparently having discharged their contents into the yolk-sac cavity (Pl. 1, figs. 1, 2). The entoderm is covered by the mesodermal cells. The amount of this tissue present in different areas of the yolk sac is subject to wide variation. Close to the embryo it is composed of flattened cells which form a definite layer, the splanchnic mesothelium, which is intimately related to the underlying entoderm. In other areas the two layers are separated by mesenchymal tissue; in this tissue, and in the spaces enclosed by it, lie haemocytoblasts and other cells. At the abembryonic pole it is composed of many layers of cells in which are seen various stages in the formation of endothelial-lined spaces and developing blood cells (see under Angiogenesis, p. 29).

Amnion

The dimensions of the amnion are 1·35 by 1·34 mm., and its height is 0·32 mm. It extends caudally to form a diverticulum which leads into the connecting stalk (Text-figs. 2, 3). The lining cells of the amnion are flattened and elongated in type except where it comes into contact with the mesoderm of the connecting stalk; here the cells are cuboidal and the surface is irregular. The nuclei of the cells are near the amniotic cavity. Some degenerated ectodermal cells and cell detritus are found in the amniotic cavity. The free surface of the amnion, in the greater part of its extent, is covered with rather flattened mesodermal cells. In places the mesodermal cells are raised to form small blebs or vesicles.

ANGIOGENESIS AND HAEMOPOIESIS

The early stages of haemopoiesis in the human subject have recently been investigated by Bloom & Bartelmez (1940), who have distinguished the following morphological grades in the development of corpuscles and defined them under the following names:

(1) Haemocytoblasts.
(2) Primitive blood cells.
(3) Lymphoid wandering cells.
(4) Primitive erythroblasts.
(5) Definitive erythroblasts.
(6) Definitive erythrocytes.

Additional types of cells are described by them in both intravascular and extravascular zones of haemapoietic organs or tissues, namely macrophages, giant cells, megakaryocytes, and granular leucocytes.

The characteristics of these cells are defined by Bloom & Bartelmez as indicated below, and as the characteristics agree closely with those which we have observed in the Shaw embryo, we propose to adopt the nomenclature which they have employed, in so far as the types described are encountered at the stage of development which has been reached by the Shaw embryo. In adopting this nomenclature, however, we do not wish it to be inferred that we use the names in any other than a descriptive sense, and also it should be understood that by so doing we do not associate ourselves with certain current theories as to the pluripotentialities of some of the stem cells which are described.

Some of the principal features of the types as defined by Bloom & Bartelmez are:

(1) *Haemocytoblasts.* 'Spherical, or slightly polygonal cells, often provided with amoeba-like processes' which often occur intra- and extravascularly. 'The cytoplasm is deeply basophil and may contain a few vacuoles.' 'The relatively very large nuclei are spherical or slightly indented.' 'The very large and acidophil nucleoli may be single or multiple and are often very angular.' 'The finely granular chromatin is aggregated in larger masses along the nuclear

membrane in some cells.' 'These cells are identical in appearance not only with the primitive blood cells but also with the large, free, basophil stem cells seen in all haematopoietic foci of both embryonic and adult man.'

(2) *Primitive blood cells.* 'These are the first free precursors of blood cells and are morphologically identical with haemocytoblasts' (which may be found in blood-forming centres in older embryos, foetuses, and adult man).

(3) *Lymphoid wandering cells.* 'This name was given by Maximow (1909) to cells of lymphocyte, that is haemocytoblast, type, which he found moving in the mesenchyme.'

(4) *Primitive erythroblasts.* 'This is the largest group of cells in the blood-cell forming areas. The youngest ones closely resemble, and are connected by many transition forms with, the haemocytoblasts. The nuclei become smaller, the nucleoli smaller and often more numerous, and the chromatin particles more prominent as the cells mature. The cytoplasm remains about the same size but loses its basophilia, the colour gradually changing as haemoglobin accumulates within it.'

(5) *Definitive erythroblasts* (e.g. those found in older embryos (20 mm.) in the wall of the yolk sac, and other situations in foetal and adult life, and which are commonly known as normoblasts). 'The cytoplasm becomes paler and the chromatin assumes a more regular distribution, with the nucleoli smaller and more numerous; this is often spoken of as the checker board nucleus.' 'The nucleus becomes progressively smaller and pycnotic and is finally extruded to produce the mature definitive erythrocyte.' Proliferation by mitosis occurs in definitive erythroblasts as in primitive erythroblasts.

(6) *Definitive erythrocytes.* The presence of erythrocytes is first mentioned in an 18-somite human embryo (H.E., H. 1516) in which a few extravascular primitive erythrocytes, singly or in pairs, were noted in the yolk-sac mesoderm. The greater number of the developing blood cells seen at this stage were, however, intravascular haemocytoblasts, and primitive erythroblasts. (In this embryo it is noted that the circulation had commenced and the authors state that the vessels of the villi contain polychromatophil primitive erythroblasts at various stages of maturity and an occasional haemocytoblast; and further, that there was no haemopoiesis in the mesenchyme of the villi.)

A few words of explanation are perhaps necessary here in order to distinguish the exact meaning which is implied by the use of the qualifying adjectives 'primitive' and 'definitive'; thus the primitive blood cells are stated to be 'morphologically identical with haemocytoblasts'—the difference here lies in the circumstance that the primitive blood cells are the first haemocytoblasts to appear whereas the name haemocytoblast is a more general term than primitive blood cell, and is applicable to all developing blood cells having the special characters of haemocytoblasts (e.g. basophil cytoplasm and amoeboid processes) wherever and whenever these cells are recognizable. Thus they may be present in mesenchymal connective tissue, lymphoid tissues, or the medulla of adult bone; whereas the use of the term primitive blood cell is

restricted to the parent cell of the early embryonic stage when blood is commencing to be formed on the yolk sac or vascular area. The terms primitive and definitive, when used in descriptive embryology, usually denote respectively the first or undifferentiated and the final stage of development of any particular cell or organ. When, however, it is implied that successive independent generations of developing cells follow one another, there is apt to be confusion unless this is clearly stated.

Our observations on angiogenesis and haemopoiesis in the Shaw embryo will be described under the following headings:

(1) The chorionic mesoderm and villi.
(2) The connecting stalk.
(3) The yolk sac.

Chorionic mesoderm and villi

This presents the usual appearance of embryonic mesenchyme at the presomite stage of development, namely, a loose reticular tissue, formed by a delicate syncytial network of feebly stained protoplasmic strands and laminae, which enclose irregular intercellular spaces. Basophil nuclei of varying size and shape which occasionally are seen to be undergoing mitosis are present at the nodes of the network. The loose reticular stratum is limited, on its inner side, by a thin membranous layer of flattened cellular elements. This is incomplete in certain places where the chorionic mesenchyme becomes continuous with protoplasmic strands of the magma reticulare. In those places where the membrane is well developed it forms a definite mesothelium which resembles the splanchnic mesothelium covering the basal part of the body stalk and the yolk sac, but the cellular elements of the chorionic membrane are more delicate and flattened than in the yolk-sac mesothelium. Branching strands of angioblastic tissue are present throughout the whole extent of the chorionic mesenchyme and the central mesodermal cores of the villi. They are readily distinguished by their diffuse pale pink colour and finely granular cytoplasm. It is difficult to determine the exact relations of the strands but they appear to be connected with (1) the surrounding mesenchymal tissue, (2) the chorionic mesothelium, and (3) the cytotrophoblast. Vacuoles are frequently visible in the cytoplasm and, by fusion of these with one another, intracellular spaces or lumina are formed in the larger and more differentiated strands which are becoming transformed into capillary vessels and vascular sinuses bounded by an endothelial wall.

The spaces in the mesenchyme are thus of two kinds: (1) the irregular, intercellular spaces or meshes of the reticulum, and (2) vascular spaces lined by endothelium.

The vascular sinuses and developing capillary vessels are mostly empty (Pl. 3, figs. 7, 8); very occasionally, however, various stages in the 'rounding up' of the primarily flattened cell elements of the wall of a vascular sinus are seen, until the stage is reached of a round bud with a constricted neck pro-

jecting into the lumen of the sinus. Within the lumen of the sinus (Pl. 4, fig. 9), and in the vicinity of these buds, free cells having all the characters of the endothelial cell elements forming the buds are frequently visible. These liberated cells arising from the endothelium vary in size, but are on the whole smaller than the erythroblasts in the blood islands and vascular spaces of the yolk sac. Occasionally the free cells are seen to be undergoing mitosis; on the other hand, some show degenerative changes.

The vascular spaces and developing capillaries are seen in the chorionic mesenchyme and central cores of the villi throughout the whole extent of the vesicle, but spaces and capillaries containing free endothelial cells appear more frequently in the chorionic mesenchyme in the vicinity of the body stalk. The transformation of the angioblast by canalization into sinuses and vessels is more advanced on the placental side of the chorionic vesicle, and at the equator where the villi are largest, than at the abembryonic pole.

Free spherical cells, or groups of such cells, of mesothelial type are also seen in the magma reticulare, and more particularly in the neighbourhood of the body stalk.

Fibroblasts and small round cells of a lymphoid type are also occasionally seen in the intercellular spaces of the chorionic mesenchyme; the nuclei of some of the fibroblasts show mitosis.

The appearance of the angioblastic strands and vessels in the mesenchyme forming the central core of the chorionic villi is similar to that in the parietal mesenchyme, though, on the whole, the development is less advanced than it is in that layer. The development of angioblastic tissue and vessels in the villi is taking place throughout the whole extent of the vesicle but, as in the parietal mesenchyme, the largest and most differentiated vessels are found in villi near the attachment of the connecting stalk. As in the parietal mesenchyme, the vessels arise by vacuolation of the protoplasmic strands and the fusion of vacuoles to form an intracellular lumen which contains, as a rule, no formed elements. The appearance of one of these vessels and the relation of its wall to the surrounding mesenchyme are well seen in the transverse section shown in Pl. 3, fig. 8, in which it will be noted that the upper wall of the vessel, as seen in the photograph, is formed by angioblast, whereas the lower is completed by a delicate film or membrane which is continuous with both the angioblast above and with the surrounding mesenchyme. The vessels of a villus are often continuous at the base of the villus with a vessel in the parietal layer of the chorion, sometimes by a T-shaped junction suggesting outward growth into the villus of a sprouting bud from a previously formed vessel in the mesenchyme. Other strands, however, appear to be formed independently by transformation of the mesenchyme, although it is possible, as claimed by Hertig (1935), since the strands often appear to be continuous with the trophoblastic cells, that they may have arisen at an earlier stage by delamination from the cytotrophoblast, but the end-product, namely, the endothelium lining a blood sinus or capillary vessel, is so similar to that which occurs in the

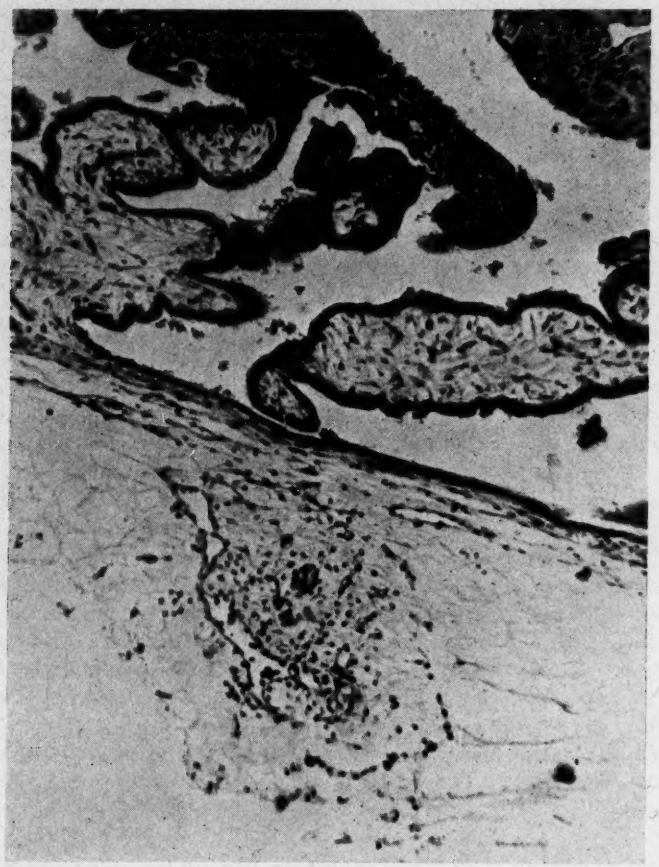
formation of the blood vessels in the yolk-sac mesoblast and other mesodermal tissues, that we are inclined to think that the vessels arise by the transformation or differentiation of mesodermal cells. When once the angioblastic tissue has become differentiated it reproduces itself by budding out processes which spread by proliferation of endothelial cells and extension, as shown by Clark & Clark (1932 and 1937) and also Clark (1909) in their accounts of observations upon the growth and extension of new blood and lymphatic capillaries from pre-existing capillaries in the liver, rabbit ear, and in amphibian and mammalian material respectively.

Connecting stalk

This region must be considered as consisting of two segments: (1) the basal part at the chorionic end of the stalk, where the supporting tissue consists of a loose mesenchymal reticulum continuous with the parietal chorionic mesenchyme, and (2) the proximal, or embryonic, end—the umbilical stalk—which encloses the allanto-enteric diverticulum and in which the supporting tissue consists of closely packed, rounded, embryonic cells continuous with the yolk-sac mesoblast. In the former (Text-fig. 10) a large capillary vessel is seen which extends into the parietal mesenchyme. Its endothelial wall shows bud-like projections into the lumen, and some free rounded cells lying in the lumen. These cells are smaller than the haemocytoblasts and early erythroblasts found in the blood islands in the wall of the yolk sac (cf. Pl. 4, fig. 9, with Pl. 4, figs. 10, 11), and they differ considerably in appearance, having compact deeply stained spherical nuclei surrounded by a relatively small amount of cytoplasm. In contrast with these isolated cells, definitely arising by a process of budding from a preformed endothelial wall, are the cells found in the region of the allanto-enteric diverticulum, which lie in the mesoblast in close relation with the entodermal wall of the diverticulum. These cells closely resemble those of the blood islands of the yolk sac, with which they are strictly homologous, and with the blood-vascular system of which they will later become continuous, when an endothelial wall has been differentiated from the mesoblast surrounding them, and the two systems become joined. A connexion is also formed, at a later stage, of the plexus of vessels which gives rise to the umbilical arteries and the umbilical vein with the independently formed vessels in the chorionic segment of the stalk. The time at which this occurs appears to be variable; thus, in Ingalls's human embryo at the beginning of segmentation (three somites) the author (1921) states that 'the distal portion of the stalk is occupied by a few small blood vessels through which a narrow connexion is set up with the vessels of the chorion'.

In contrast with this we find in Stieve's 13½-day human embryo Hugo (1926) —total length of embryonic rudiment 0·75 mm. and length of embryonal shield 0·57 mm.—the development of solid blood islands and blood vessels in the stalk unusually far advanced considering the early stage of development of this embryo. Stieve regards the mesoderm which contains the blood islands as

arising from the 'sickle node' and, therefore, axial in origin. He also believes that the cells forming the islands in the stalk, like those of the islands in the yolk sac, may originate, in the human subject, from both entoderm and



Text-fig. 10. The photograph shows the junction of the connecting stalk with the chorionic mesoderm, and the advanced stage of development of the vessels in the stalk, adjacent parietal mesenchyme, and mesenchymal tissue of the villi. In the cavity of the chorionic vesicle isolated groups of degenerate mesenchymal cells and free nuclei are visible. The connexions of the villi with the decidua, and of the intervillous spaces with the basal sinus, are also clearly shown. Section no. 144. ×100.

mesoblast, as in other species of mammalian embryos. The mesenchyme at the chorionic end of the stalk, and the chorionic mesenchyme, which contain blood vessels but no islands, are regarded by him as 'morula mesoderm', which

difference, we believe, may explain the difference in type of angiogenesis in the two parts of the stalk. It is not until the vessels are fully formed in both parts and are connected that, when the heart commences to beat, blood flows from the umbilical arteries into the chorionic or placental vessels.

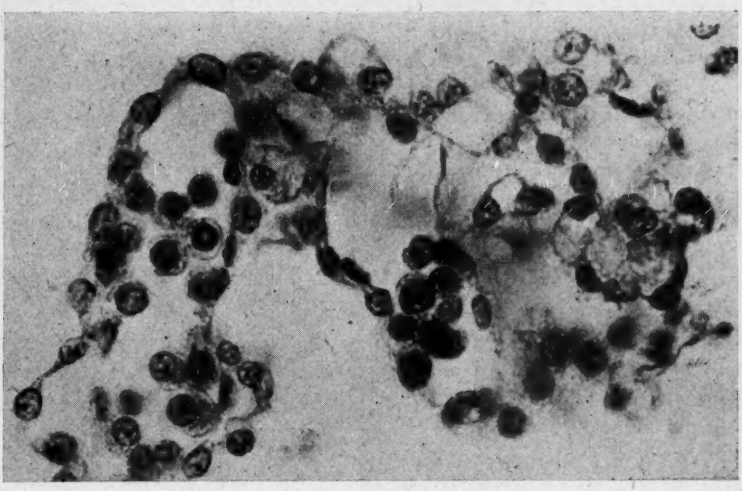
Yolk sac

There are three types of blood island on the surface of the yolk sac:

(1) Haemocytoblasts and early primitive erythroblasts not enclosed in an endothelial covering.

(2) Solid masses of haemocytoblasts enclosed in an endothelial wall.

(3) Endothelial-lined cysts or vessels containing a few free haemocytoblasts and other cell types.



Text-fig. 11. The photograph shows the early formation of intercellular and intracellular spaces. Some of the intercellular spaces are lined by entoderm (upper layer in the photograph) on one side and by mesenchymal tissue on the other. Some haemocytoblasts and primitive erythroblasts (cells with clearer cytoplasm) are lying free in the spaces. Section no. 46. ×560.

Intermediate stages between these three types of island are frequent, and intercellular spaces not lined by endothelium are also present in the mesoblastic tissue between the splanchnic mesothelium and the yolk-sac entoderm (Text-fig. 11).

Angiogenesis and haemopoiesis are most advanced at the distal or abembryonic pole of the sac (Pl. 1, fig. 1) where, presumably, the blood islands first commenced to be formed in the zone corresponding to the vascular area of lower types of Mammalia; these two processes are less advanced in the circumferential zone near the margin of the embryonic disc where the earlier stages

of smaller solid islands not yet enclosed in endothelium are best studied (Pl. 1, fig. 2). In this region the yolk-sac wall consists of two well-defined layers, the entoderm and splanchnic mesothelium, between which in the loose mesoblastic tissue are empty spaces, some of which are interstitial and others are lined by endothelium. There are also small solid masses of proliferating cell elements, haemocytoblasts and primitive erythroblasts, which represent the earliest stage of blood formation (Pl. 4, fig. 10). Sometimes the blood cells project from one side into the lumen of an endothelial-lined space into which at a later developmental stage the freed blood cells will be discharged (Pl. 4, fig. 11). The cell outlines of the future haemocytoblasts are, in the earliest stages, absent or only very indistinctly visible (Pl. 1, fig. 2). In the more advanced stages the cell elements of these plasmodial masses differentiate and become separated from each other and are finally liberated in the form of free haemocytoblasts showing irregular protoplasmic processes. These by further division form the first generation of embryonic red blood cells or primitive erythroblasts. Some of the cells show signs of degeneration, e.g. the formation of vacuoles, the contents of which when discharged may contribute to the formation of the embryonic plasma.

In the later stages of development seen at the abembryonic pole of the yolk sac, when the endothelial walls of the blood spaces are completed (Pl. 1, fig. 1), the vessels are much increased in size and communicate with each other, forming the capillary plexus from which the vitelline vessels will afterwards be formed in the early stages of somite development, as has been demonstrated in the Ingalls (1921) three-somite human embryo. In these vessels we can recognize the following types of cell (Pl. 1, fig. 1): haemocytoblasts, transitional stages between these and primitive erythroblasts, primitive erythroblasts, intermediate erythroblasts, late primitive erythroblasts, degenerating erythroblasts, non-nucleated primitive erythrocytes, and binucleated giant cells.

DISCUSSION

It is proposed to compare the present embryo only with those that are nearly related to it in development. It is at approximately the same stage of development as Wa 17 (Grosser, 1931) and would, therefore, be at a later stage than the Manchester embryo (Florian & Hill, 1935) and younger than the Peh_1-Hochstetter (Rossenbeck, 1923).

If we accept the patient's history of coitus, the embryo has a coital age of 26 days, much older than the corresponding embryos of this stage of development. The embryo, indeed, appears to be much younger than its supposed coital age, being at about the 17th or 18th day of development, and as it was procured as the result of an operation there is no reason to suppose that it had been dead for some time.

Embryonic disc

After comparing the shape of the dorsal views of graphic reconstructions, Hill & Florian (1931) have suggested that the embryonic discs of embryos may be divided into two varieties, as Rabl (1915) has done for the rabbit, a narrow type, e.g. the embryos Bi 24 (Florian, 1934), Manchester (Florian & Hill, 1935), Wa 17 (Grosser, 1931), and Dobbin (Hill & Florian, 1931), and to these may be added the embryos, Kl 13 (Grosser, 1913), Ingalls (1918), Carnegie 5960 (Heuser, 1932), and HR_1 (Johnston, 1940), and a broader type, e.g. the Hugo (Stieve, 1926), Scho (Waldeyer, 1929) and Peh_1-Hochstetter (Rossenbeck, 1923) embryos; to this list may be added the Thompson-Brash (1923) and HO (Fahrenholz, 1925) embryos.

The embryo Shaw would belong to the latter group. In this connexion it may be pointed out that the head process of this embryo is at an early stage of development, and this may partly account for the fact that the disc is broad; on the other hand, the Peh_1-Hochstetter embryo has a well-developed head process.

The distinct dip on the embryonic disc in front of Hensen's knot is a feature of this embryo, and it is partly due to the proliferation of the cells at the node. In the HO embryo (Fahrenholz, 1925) there is also a distinct elevation at the knot. In the Scho embryo (Waldeyer, 1929) there is an elevation in front of Hensen's knot and the disc slopes gently away towards the anterior edge. The Carnegie 5960 (Heuser, 1932) embryo shows a distinct slope some distance in front of the node. There is no marked convexity of the disc in the sagittal plane as described in HR_1 by Johnston (1940); this author is of the opinion that this convexity is the result of abnormal growth. The concavity in the Shaw embryo, in our opinion, is normal and is the result of the rapid proliferation at Hensen's knot. It may, however, be an artefact due to fixation. The arrangement of the cells in the ectoderm is typical for an embryo of this stage of development.

Primitive streak, cloacal membrane, allanto-enteric diverticulum and yolk sac

The arrangement of the layers in the primitive streak is essentially similar to that described in embryos of a corresponding stage of development and will not be discussed further.

The cloacal membrane is easily recognized in this embryo. It involves the posterior part of the embryonic disc and the proximal part of the allanto-enteric diverticulum. The junction between the two parts makes a distinct angle as in the Peh_1-Hochstetter (Rossenbeck, 1923) embryo. There is no indication of the separation of the cloacal membrane into two parts by the appearance of mesoderm between ectoderm and entoderm as described by Florian (1930) in the Bi II and Šternberg (1927) embryos, and by Wyburn (1937) in the McIntyre embryo. The present embryo does not throw any light

on whether the cloacal membrane first appears between the amniotic ectoderm and entoderm, as in the Beneke embryo (Florian & Beneke, 1930–1) and HR_1 embryo (Johnston, 1940), or within the embryonic disc, as in the Bi I and Fetzer embryos (Florian, 1933).

The proliferation of the ectoderm which gives rise to the primitive streak in the Edwards-Jones-Brewer embryo (Brewer, 1938), which is well preserved, seems to us to settle the controversy as to whether the primitive streak appears before or after the cloacal membrane. In the Brewer embryo the primitive streak is at the earliest stage described so far for the human subject, and there is no indication yet of the cloacal membrane. Further, in this embryo the primitive streak is a crescentic mass, as in the pig (Streeter, 1927) and the ferret (Hamilton, 1937), and it lies at the posterior part of the disc and not at some distance in front of the edge of the disc as shown by Florian (1933) for the OP and WO embryos of von Möllendorff, and the Fetzer embryo.

The mesodermal cells which arise from the posterior part of the primitive streak in the Shaw embryo pass around the cloacal membrane to the connecting stalk. The cells are closely packed together as described by Florian & Beneke (1930–1), and Hill & Florian (1931) in the Dobbin embryo.

It is not our intention to enter into a detailed discussion on the developmental history of the cloacal membrane since, in the present embryo, it is already well developed. We are, however, unable to accept the interpretation of Florian (1933) and Wyburn (1937) for this structure. Florian states: 'If we realize that the cloacal membrane and the primitive streak represent parts of the blastopore in lower vertebrates, we must accept the view that the homologue of the blastopore in Man originates in two separated areas which grow together only during later development. This fact has perhaps caused the difficulties in interpretation of the structure which I regard as cloacal membrane.' Wyburn writes: 'The appearance of the primordium of the primitive streak representing the fused lips of the blastopore acclaims the formation of secondary mesoderm separating the ectoderm and entoderm in this region', and 'Caudal to the fused lips of the blastopore and reaching to the attachment of the stalk remains a relatively extensive area of contact, the anlage of the cloacal membrane....The cloacal membrane would thus be the homologue of the ventral lip of the blastopore and adjacent area.' There is apparently no phylogenetic reason why the cloacal membrane should be regarded as part of the blastopore. In *Amphioxus* and Amphibia the blastopore is recognized as an invagination and the cells around it are proliferating rapidly to give rise to axial structures and mesoderm. In reptiles, birds and mammals the ventral lip of the blastopore is represented by the primitive streak and, as such, gives rise to mesoderm. The cloacal membrane in man and mammals, on the other hand, is a region where ectoderm and entoderm are in contact, or actually fused, and gives rise neither to axial structures nor to mesoderm. It is difficult, therefore, to see why it should be regarded as part of the primitive streak or of the blastopore. If Rabl's (1915) opinion is accepted, that gastrulation (formation

of head process and mesoblast) is a process of growth which allows certain presumptive organs to reach their definitive positions, then it seems to us that the cloacal membrane must be regarded as lying outside this field of growth. We are rather of the opinion of Keibel (1896) that the cloacal membrane is a secondary structure. The matter is only to be decided by experimental methods as it has been in lower vertebrates. Whether the area in the connecting stalk where mesoderm is in contact with the amniotic ectoderm can be regarded as part of the blastopore, as it gives rise to mesoderm, is another question. The origin of the primitive mesoblast from trophoblast, as described by Hertig (1935), introduces further and fundamental difficulties in accepting the view that the area described by Florian is part of the blastopore.

Notochordal process and chorda canal

The presence of a chorda canal has been described in embryos at approximately the same stage of development as the present embryo.

The youngest embryo described with a head process (length 0·06 mm.) is the Meyer (1924) embryo; according to Florian (1928) the structure identified as the head process was erroneously interpreted. Johnston (1940) describes a head process (length 0·04 mm.) consisting of a mass of cells derived from what he interprets as Hensen's knot in the HR_1 embryo. It should be pointed out that Florian does not recognize a head process in the HR_1 embryo (see Johnston's paper). As far as can be ascertained from the photographs published in Johnston's paper we are of the opinion that this author's interpretation is the correct one. One of us (Hamilton, 1937) has found appearances similar to that described and figured by Johnston in the early stages of the development of the head process in the ferret.

An undoubted head process is present in the embryos H. Schm. 10 (Grosser, 1931), Hugo (Stieve, 1926) and Bi 24 (Hill & Florian, 1931; Florian, 1934). In the Hugo embryo the process (length 0·07 mm. according to Hill & Florian (1931), and 0·09 mm. according to Stieve) is composed of irregular cells which are never columnar; they are, however, in continuity with the intra-embryonic mesoderm. The entoderm is missing underneath the head process. In the H. Schm. 10 the head process (length 0·1 mm.) is composed, in one section, of columnar cells which surround a space which Grosser recognized as the chorda (Lieberkuhn's) canal. Thus at an early stage the cells are typical of later stages of development when a well-defined canal is established, as in the embryo described by Heuser (1932). In the Bi 24 embryo the head process (length 0·1 mm.) is more differentiated than in the Hugo. It consists of a thickened median cord and two thinner lateral wings of mesoderm. The underlying entoderm is indistinct and is represented in some places only by scattered cells, many of which have pycnotic nuclei. The pycnosis of the nuclei, in our opinion, may be regarded as the first sign of the disappearance of this entoderm. The median cord of cells ends in continuity with thickened entoderm which is recognized as the prochordal plate. The next stage in the development of the

head process (length 0·12 mm.) is found in the Manchester embryo (Florian & Hill, 1935); it has a definite Hensen's knot extending forward from which is a short head process which terminates in the prochordal plate; no mention is made of the presence of a canal in this head process. In the embryo Wa 17 (Grosser, 1931) there is the beginning of a chorda canal in the head process (length 0·18 mm.). Whether a ventral opening connects the chorda canal with the yolk sac appears to be doubtful. In the Shaw embryo, with a head process 0·18 mm. in length and, therefore, at approximately the same stage of development as the Wa 17, the dorsal opening of the chorda canal and the caudal part of the canal are distinct. We have not been able to find a ventral opening into the yolk sac. If entoderm is underlying Hensen's knot and the head process it is indistinguishable from the tissue of the knot and head process. In the embryo Thompson-Brash (1923) the head process (length 0·3 mm.) has not yet acquired a lumen, but the dorsal cells are arranged fanwise so that the appearance of a lumen is imminent. In the Peh_1-Hochstetter embryo (Rossenbeck, 1923) the head process (length 0·6 mm.) has a distinct canal. There is some doubt as to whether there are one or more ventral openings into the yolk sac (see Hill & Florian, 1931). In the Kl 13 embryo (Grosser, 1913) the chorda canal (length 0·2 mm.) communicates by means of several openings with the yolk sac. This embryo is smaller, but apparently more differentiated, than the Peh_1-Hochstetter, as is also the Dobbin embryo (Hill & Florian, 1931). There is, therefore, considerable variation in the time of appearance of the canal. It is distinct in the Shaw embryo, with a head process length of 0·18 mm., and has not yet appeared in the Thompson-Brash (head process length 0·3 mm.). There is also variation as to the stage of development when it communicates with the yolk sac.

The line of demarcation between the posterior edge of Hensen's knot and the anterior part of the primitive streak can be recognized in the present specimen. The cells in the roof of the chorda canal are tall and columnar, with clear cytoplasm towards the lumen, and are, therefore, typical for embryos of this stage of development (compare Rossenbeck, 1923; Heuser, 1932; Johnston, 1940). The obliquity of the section in the present embryo does not permit us to give an opinion as to the amount of mesoderm, if any, arising from the side of the head process. That the head process becomes intimately connected with the underlying entoderm in man is, however, borne out by the present specimen.

The prochordal plate

There is now an extensive literature dealing with the prochordal plate in man and in mammalian forms (Hubrecht, 1890; Bonnet, 1901; Adelmann, 1922; Bryce, 1924; Hill & Tribe, 1924; Hill & Florian, 1931; Heuser, 1932; Hamilton, 1937; and others).

In the present embryo the thickened entodermal cells form a horseshoe-shaped plate resembling somewhat that described by Florian & Beneke (1930–31) in the Beneke embryo. The anterior tip of the head process lies between the

two horns which project backward. The chromophilic granules first described by Bonnet (1901) in the prochordal (completion) plate in the dog have been recognized in the cells of the entoderm in that part of the disc that we interpret as prochordal plate. We have not found pouches or 'cell groups' in the prochordal plate, as described by Bryce (1924) in the McIntyre embryo (Sternberg, 1927; Heuser, 1932).

Connecting stalk

As stated earlier, we have followed Florian (1930) in recognizing two parts in the connecting stalk, an amnio-embryonal and an umbilical part. We find in the Shaw embryo, as in nearly all human embryos at this stage of development, that the amniotic cavity runs into a distinct amniotic tip at its dorsocaudal part where it comes into contact with the mesoderm in the basal part (i.e. near the chorion) of the umbilical stalk. In some of the cells of the mesoderm, entoderm, and ectoderm in the anterior part of the umbilical stalk there are conspicuous chromatic granules. Florian regards these granules as a sign of degeneration in the cells. After discussing at some length the changes occurring in the stalk tissue he comes to the conclusion that the amniotic cavity extends backwards into the umbilical stalk at the expense of the tissue of the stalk, as first described by von Möllendorff (1921), and that the cell dissolution is a preliminary to this extension.

We find that there is some irregularity of the ectodermal amniotic cells in the front of the umbilical stalk which would support the hypothesis of backward extension; we did not find, however, the sequestration of a large mass of mesoderm as described by Florian.

The vascular primordia found in the stalk are discussed under angiogenesis.

Allanto-enteric diverticulum

The allanto-enteric diverticulum in the present embryo is found to be associated at its tip with a solid mass of cells which we interpret as entodermal.

In the Mateer embryo Streeter (1920) describes the allantois as separated into two parts. In the proximal part of the distal separated portion there is a lumen. In the Sternberg embryo (Sternberg, 1927) a separated epithelial vesicle is associated with the allantois. Hill & Florian (1931) found, in the Dobbin embryo, that the terminal part of the allantois passed into a terminal vesicle. We are in agreement with the opinion expressed by them that the allantois may undergo separation into two parts during development, the distal part being subject to early degeneration. In the present specimen the cavity, if ever present, has disappeared.

Yolk sac

The details of the histological appearances of the cells in the yolk sac are discussed under Angiogenesis. The formation of the foregut by anterior, and partially by lateral, foldings is clearly shown. A folding is shown in the Heuser

(1932) embryo but the formation is regarded as a fold in the yolk sac; Heuser states that a recognizable foregut has not yet developed.

Angiogenesis and haemopoiesis

The study of this specimen affords evidence in support of certain conclusions which have previously been reached from observations made on other human embryos of approximately the same age and stage of development. It also throws light upon the following problems which are still *sub judice*:

(1) The origin of the angioblastic tissue of the primary or chorionic mesoderm.

(2) The origin of the first haemocytoblasts and primitive blood cells.

(3) The relation of the blood islands of the yolk sac and umbilical segment of the body stalk to the endothelial walls of the vessels which surround them.

(1) *The origin of the angioblastic tissue of the primary or chorionic mesoderm.* It is almost universally accepted that both blood cells and blood vessels arise from the mesenchyme. This view is based chiefly on the observations of Maximow (1909, 1927), Bloom (1938) and Bloom & Bartelmez (1940).

It seems best, in our opinion, to approach the subject of the early stages of blood-cell formation and development of blood vessels in the human subject and other vertebrate animals by an examination of the first stage of the differentiation of these elements rather than to work backward from later stages of blood formation, when differentiation of other cells from morphologically similar cells is taking place at the same time in different situations. Apart from the difficulty of deciding whether cells are developing intra- or extra-vascularly, the examination of later stages is complicated by the fact that there is a mixing in the general blood stream of cells which may be derived from different sources.

This difficulty has to a large extent been overcome by Stockard's (1915) experimental work on the origin of blood and endothelium in *Fundulus* larvae. He concludes that the origin of the blood cells and endothelial lining of the heart, aortic arches and dorsal aortae is independent. Stockard succeeded in rearing *Fundulus* larvae in weak solutions of alcohol; although a heart and intra-embryonic blood vessels lined by endothelium were developed in these larvae, the connexion of the vitelline veins with the heart was never established and consequently no circulation of either blood cells or plasma could take place. Moreover, the blood developed in the intermediate cell mass at the posterior end of the embryo, and, found in the blood islands in the vascular area of the yolk sac, remained in its original situation and was not drawn into the heart and intra-embryonic vessels. Furthermore, neither the heart with its endothelial lining nor any portion of the aortae were, at any stage of development, seen to contain an erythroblast or erythrocyte. Stockard believes that the blood islands in *Fundulus* embryos are formed by wandering mesenchymal cells which migrate from the intermediate cell mass which lies at the posterior end of the embryo, between the notochord and the caudal end of the gut; he

further states that the only mesodermal portion of the yolk sac in *Fundulus* is made up of disconnected wandering mesenchymal cells, some of which group themselves to form the blood islands, others give rise to the yolk vessel endothelium, and still other wandering cells develop into the chromatophores. Stockard thus ranges himself alongside those authors, such as Maximow, Bloom and Bartelmez, who believe that both erythroblasts and endothelial cells arise from the mesenchyme. He does not, however, believe that endothelial cells once differentiated are capable of producing erythroblasts and erythrocytes, since, in his series of embryos which developed without a circulation, he never found that the endothelial-lined heart or the vessels leading from it, such as the ventral aorta, aortic arches and dorsal aortae, contained developing blood cells; nor does he believe that the mesenchyme in general, that is to say in other places than the known haemopoietic foci, gives origin to developing red corpuscles.

In the chorionic mesenchyme, including that of the villi and that at the chorionic end of the connecting stalk, in the human subject, specialized angioblastic tissue appears at an early stage of development. This tissue gives rise, by vacuolation of primarily solid protoplasmic strands, to endothelial-lined spaces and vessels which before the circulation is established contain no primitive blood cells, although these are abundant in the blood islands of the yolk sac and are present in the umbilical stalk in the vicinity of the allantoenteric diverticulum. The formation of vessels in the chorionic mesenchyme has been specially studied in primates and in the human subject by Hertig (1935). He concluded that there is 'a simultaneous origin of angioblasts and primary mesoderm by a process of delamination and differentiation from the chorionic trophoblast of macaque and human ova', and that the isolated vascular primordia thus formed soon possess the power of independent growth and lumen formation, resulting not only in the vascularization of the chorion and primary villi but of secondary villi as well. Further, after discussing the question of haemopoiesis in the chorionic blood vessels of early human embryos, such as the Mateer (Streeter, 1920) and the Heuser (1932) presomite specimen, Hertig points out that 'blood elements begin to appear in the chorionic and villous circulation with regularity only about the time the embryonic circulation is becoming functionally established'. The generally empty condition of these chorionic vessels which we have observed in the Shaw embryo, and which was also noted by Stieve (1926) in his 13½-day human embryo, confirms Hertig's conclusions and, in the light of Stockard's experimental work, is significant. It may be noted here that the few formed elements, which appear to be arising from the endothelium of the vessels shown in Pl. 4, fig. 9, differ considerably in size and character from the primitive blood cells and erythroblasts formed in the blood islands of the yolk sac, and although they may be interpreted as being a generation of developing red blood cells arising from the endothelium of vessels which have been developed in the chorionic mesenchyme at the base of the connecting stalk, it is by no means certain that

they are such. Apart from this question it appears that angioblastic tissue is differentiated at a very early stage from the mesenchyme in which it lies, and which arises by delamination, either directly or indirectly, from an ectodermal layer, namely, the cytotrophoblast.

(2) *The origin of the first haemocytoblasts and primitive blood cells.* The results of an investigation of the early stages of development of the blood vessels and of the heart were published by Wang (1918). This research was on ferret embryos and commenced with the stage in which the embryo measured 1·15 mm. and in which no intra-embryonic blood vessels could be recognized, nor any indication of a heart rudiment. In this paper not only are the earliest stages of development of the vessels, heart and pericardium described, but there is a description of the author's observations on the earliest stages of development of the blood cells, preceded by a discussion of the literature on the origin of blood vessels and blood cells. This discussion is so complete that it will be quite unnecessary to do more than refer readers to Wang's paper and we need only give a summary here of some of his conclusions. At that time there was a diversity of opinion with regard to the germ layer from which the vascular endothelium and blood cells arise. In 1887 Ziegler expressed the view that 'the system of blood vessels and that of the lymphatic vessels are produced in their first fundaments from remnants of the primary body cavity (blastocoel) which at the general distribution of the primitive tissue (mesoderm) remain behind as vessels'. This view, therefore, harmonizes with Bütschli's hypothesis that in all Metazoa the blood-vascular system originates from the blastocoel. This theory, which may be referred to as the origin of the blood-vascular system from the primary mesoderm, has received a considerable amount of support from recent writers such as Hertig (1935) although, as mentioned under the first heading of our discussion, Hertig limits his deductions to the origin of the blood vessels (angioblast) only, and he traces that back to the simultaneous origin of the vascular rudiments and the chorionic mesenchyme from the cytotrophoblast by a process of delamination. It is worth while here to draw attention to the statement made by Wang 'that in no case has an author stated that the blood cells and vascular endothelium are derived from different germ layers', and he furnishes evidence, based on his observations on early ferret embryos, in support of his opinion that 'whilst blood cells and vascular endothelium are closely related to each other, and are found invariably between the mesoderm and endoderm, there is evidence to show that, in the ferret, the origins of these two vascular elements are separate and distinct—the blood cells arising from the endoderm, and the vascular endothelium from the mesoderm'. 'Blood cells develop at first extra-embryonically in the area vasculosa in the form of clusters of spheroidal cells which are provided with large and round nuclei and with a comparatively small amount of protoplasm. These are for the most part found adherent to the endoderm in the neighbourhood of their origin, before they are engulfed by the endothelium, and are in most instances identical in structure with the endodermal cells

where the contact is intimate.' 'On the other hand, the cells which form the endothelial rudiment are mesodermal in origin.'

Wang's microphotographs of sections of early ferret embryos show features which are very similar to those of the early stages in the development of the blood islands in the presomite phase of human embryos, and we believe that it may be safely concluded that the process of blood formation is essentially the same in the ferret as it is in man, and that any differences in the conclusions which may be drawn are due to differences in interpretation rather than to differences in species.

Another supporter of the entodermal origin of the first embryonic blood cells is Piney (1927), who bases his argument in its favour on the following facts: (1) 'at the time of earliest blood formation there are no formed organs in the embryo and, therefore, transport of the yolk substances is very essential'. It may be noted that in the Shaw embryo vacuoles are frequently visible in the cytoplasm of the early blood cells as well as of the cells forming the walls of the blood islands and the entodermal cells: (2) 'The endodermal masses become surrounded by mesodermal endothelium which, therefore, takes no part in this form of haematopoiesis.' (3) It is very striking that this extra-embryonic form of haematopoiesis is later seen in the liver of the embryo.' With reference to Maximow's claim that these first-formed blood cells are the ancestors of all the types that develop later, Schridde and Piney contend that 'they are all haemoglobiniferous elements'. Maximow holds that some of these primitive cells become haemoglobiniferous and act as temporary carriers of oxygen (primitive erythroblasts), while others retain all their haemopoietic potencies, whereas Piney considers that 'these first formed cells have only a short life in the embryo but that they are all nucleated red cells (megaloblasts) which normally persist longest in the liver of the embryo from which they disappear before birth'. Piney agrees with Sabin (1920) that certain amoeboid cells develop from, or near, the primitive endothelium, and quotes Ferrata as stating that 'all the *white* cells of primitive blood in the embryo are of such endothelial origin (haemohistioblasts), i.e. are not derived from the first formed intravascular islet cells'. The marked difference in the shape and character of the nuclei belonging to the early or primitive blood cells in the yolk sac of a human embryo from those of the endothelial cells of the vessel which encloses them is evident in his figure showing mitoses of the blood cells. The nuclei of the blood cells are rounded in form, whereas those of the endothelial cells are elongated and spindle or lens-shaped. The difference is seen to be even more pronounced in the figure showing the cross-section of a vessel of the area vasculosa of a rabbit embryo (five somites) by Maximow & Bloom (1934), who, however, explain the difference as being due to rounding off of the endothelial cells and their transformation into primitive blood cells. This 'rounding off' of cells which are afterwards set free in the lumen of the vessel, as seen in Pl. 4, fig. 9, certainly appears to take place in the mesenchyme at the chorionic end of the connecting stalk, and the appearances seem to support the

view expressed by Piney that 'the embryo is supplied for a short time with two varieties of blood; one derived from the entoderm, and composed purely of haemoglobiniferous cells, and the other of mesenchymal origin and consisting of both red and white cells'.

Quite recently a description of haemopoiesis from the clinical standpoint has been published by Gilmour (1941). He has made a special study of early presomite and somite embryos prior to the establishment of the circulation, as well as of older embryos, foetuses, and infants up to 21 days after birth. From his study of the earlier stages of development he concludes that 'vessels arise from mesodermal cells independently in three areas, the yolk sac, the chorion—perhaps at first limited to the body stalk—and the embryo. The vessels in each of these areas unite to form nets or systems. The three systems later unite with each other and the complete circulation is established.' 'Blood islands form constantly in the yolk sac and consist of separate vascular units containing blood cells. The cells are first haemocytoblasts which arise from the vessel wall. Later, the cells are almost entirely primitive erythroblasts but a few haemocytoblasts persist and a few differentiate into histiocytes.'

It may be noted here that this account differs from the usual text-book description in which it is stated that the primarily uniform mass of rounded cells which forms a 'blood island' or 'angioblastic cord' in the earliest stage of development becomes differentiated into a superficial layer of flattened cells—the endothelial wall of the blood vessel—which becomes separated by a cleft containing embryonic plasma from the central cells, which retain their rounded form and become the primitive blood cells.

Space will not permit us to review adequately this paper as a whole; we may state, however, that we are in agreement with Gilmour's conclusions regarding the independent development from mesoderm of vessels in the three areas mentioned and their subsequent union prior to the establishment of the circulation. We cannot, however, wholly concur with his statement that the first haemocytoblasts arise from the vessel wall, as there are numerous instances of small islets which are not enclosed in an endothelial wall, the cells of which cannot have arisen from differentiated endothelium by transformation, although, as Piney suggests, this does not exclude the formation of other cells of a different type from this source.

(3) *The relation of the blood islands of the yolk sac and the umbilical stalk to the endothelial walls of the vessels which are formed around them.* The frequent appearance of syncytial masses, or small groups of rounded cells, which lie in the interval between the entoderm and the mesoderm of the yolk-sac wall (Pl. 4, fig. 10), and which are either isolated or in intimate relation with the entoderm, and also of spaces which are bounded, on the inner side, by a single layer of entodermal cells (Text-fig. 11), and, on the outer side, by undifferentiated mesenchyme, suggests that the cells which comprise these groups, and which are often indistinguishable from the entodermal cells, may be primarily

derived from the entoderm. The vessel wall, on the other hand, appears to be developed by differentiation of the neighbouring mesenchyme into endothelium either before or subsequently to the formation of the island, or the primitive blood cells may grow outward as a rounded mass into a mesodermal space the wall of which is simultaneously becoming transformed from undifferentiated mesoblast into a continuous endothelial wall.

This embryo also presents some interesting features in connexion with the early development of the heart and its relationship to the anterior part of the wall of the yolk sac. The description and discussion of these in conjunction with the development of the pericardium, floor of the foregut, and intra-embryonic vessels, we propose to defer for consideration in a later communication.

SUMMARY

1. A description is given of a well-fixed presomite human embryo at a stage of development with a blastopore, notochordal process and chorda canal.

2. The age of the embryo is discussed in relation to embryos at approximately the same stage of development.

3. A well-defined cloacal membrane is formed which involves the posterior part of the embryonic disc and the proximal part of the allanto-enteric diverticulum.

4. A description is given of the formation of blood vessels from specialized angioblastic tissue in the chorionic mesenchyme. The majority of these vessels at this stage of development contain no blood cells.

5. The vascular spaces are partly developed by the fusion of small vacuoles, which are formed in solid angioblastic cords (intracellular spaces), and partly by direct transformation of mesodermal cells into flattened endothelium, which may either enclose the blood islands of the yolk sac, or form the walls of vascular spaces, which at first empty and incomplete, become secondarily filled with blood cells and enclosed by a continuous membrane.

6. The earliest generation of blood cells (haemocytoblasts and primitive erythroblasts) are formed in the wall of the yolk sac and in the umbilical segment of the connecting stalk in close connexion with the entoderm of the allanto-enteric diverticulum, and in the situation of the future umbilical vessels. A few rounded cells of endothelial origin were, however, found in the mesenchyme at the base, or amnio-embryonic segment, of the connecting stalk. These differ in type from the former cells which arise in close association with the entoderm of the yolk sac and its diverticulum.

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REFERENCES

Adelmann, H. B. (1922). *Amer. J. Anat.* **31**, 55.

Allen, E., Pratt, J. P., Newell, Q. U. & Bland, L. J. (1930). *Contr. Embryol. Carneg. Instn*, **22**, 45.

Bloom, W. (1938). *Handbook of Haematology*. Ed. by Downey. N.Y.: Hoeber. Vol. 2.

Bloom, W. & Bartelmez, G. W. (1940). *Amer. J. Anat.* **67**, 21.

Bonnet, R. (1901). *Anat. Hefte*, **16**, 231.

Brewer, J. I. (1938). *Contr. Embryol. Carneg. Instn*, **27**, 87.

Bryce, T. H. (1924). *Trans. Roy. Soc. Edinb.* **53**, 533.

Bütschli, O. (1882). *Morphol. Jb.* **8**, 474.

Clark, E. R. (1909). *Anat. Rec.* **3**, 183.

Clark, E. R. & Clark, E. L. (1932). *Amer. J. Anat.* **49**, 441.

—— —— (1937). *Amer. J. Anat.* **60**, 253.

Fahrenholz, C. (1925). *Z. mikr. Anat.* **3**, 249.

Flew, J. D. (1941). *Brit. med. J.* 11 Jan., p. 66.

Florian, J. (1928). *Z. mikr. Anat.* **13**, 500.

—— (1930). *J. Anat., Lond.*, **64**, 454.

—— (1933). *J. Anat., Lond.*, **67**, 263.

—— (1934). *Bratisl. lekár. listy*, **14**, 299.

Florian, J. & Beneke, R. (1930–31). *Anat. Anz.* Erg. **71**, 229.

Florian, J. & Hill, J. P. (1935). *J. Anat., Lond.*, **69**, 399.

Gilmour, J. R. (1941). *J. Path. Bact.* **52**, 25.

Grosser, O. (1913). *Anat. Hefte*, **47**, 649.

—— (1930–1). *Anat. Anz.* Erg. **71**, 135.

—— (1931). *Z. ges. Anat.* 1. *Z. Anat. EntwGesch.* **94**, 275.

Hamilton, W. J. (1937). *Trans. Roy. Soc. Edinb.* **59**, 165.

Hertig, A. T. (1935). *Contr. Embryol. Carneg. Instn*, **25**, 39.

Heuser, C. H. (1932). *Contr. Embryol. Carneg. Instn*, **23**, 251.

Hill, J. P. & Florian, J. (1931). *Philos. Trans.* B, **219**, 443.

Hill, J. P. & Tribe, M. (1924). *Quart. J. micr. Sci.* **68**, 513.

Hubrecht, A. A. W. (1890). *Quart. J. micr. Sci.* **31**, 499.

Ingalls, N. W. (1918). *Contr. Embryol. Carneg. Instn*, **7**, 111.

—— (1921). *Contr. Embryol. Carneg. Instn*, **11**, 63.

Johnston, T. B. (1940). *J. Anat., Lond.*, **75**, 1.

Keibel, F. (1896). *Arch. Anat. Physiol., Lpz.*, p. 250.

Knaus, H. (1934). *Periodic Fertility and Sterility in Women*. Vienna: W. Maudrich.

Maximow, A. (1909). *Arch. mikr. Anat.* **73**, 444.

—— (1927). v. Möllendorff's *Handb. Mikr. Anat. Mens*, **2**. Berlin.

Maximow, A. & Bloom, W. (1934). *Textbook of Histology*. London: Saunders.

Meyer, P. (1924). *Arch. Gynäk.* **122**, 38.

Möllendorff, W. von (1921). *Z. ges. Anat.* 1. *Z. Anat. EntwGesch.* **62**, 406.

Piney, A. (1927). *Recent Advances in Haematology*. London: Churchill.

Rabl, C. (1915). *Arch. mikr. Anat.* **88**, 1.

Rossenbeck, H. (1923). *Z. ges. Anat.* 1. *Z. Anat. EntwGesch.* **68**, 325.

Sabin, F. R. (1920). *Contr. Embryol. Carneg. Instn*, **9**, 213.

Shaw, W. (1925). *J. Physiol.* **60**, 193.

—— (1932). *Brit. med. J.* 5 Mar., p. 411.

Siegel, W. (1916). *Münch. med. Wschr.* **63**, 748.

Sternberg, H. (1927). *Z. ges. Anat.* 1. *Z. Anat. EntwGesch.* **82**, 142.

Stieve, H. (1926). *Z. mikr. Anat.* **7**, 295.

Stockard, C. R. (1915). *Amer. J. Anat.* **18**, 227.

Streeter, G. L. (1920). *Contr. Embryol. Carneg. Instn*, **9**, 389.

—— (1927). *Contr. Embryol. Carneg. Instn*, **19**, 73.

Thompson, P. & Brash, J. C. (1923). *J. Anat., Lond.*, **58**, 1.

VENNING, C. H. & BROWNE, J. S. L. (1937). *Endocrinology*, **21**, 711.
WALDEYER, A. (1929). *Z. ges. Anat.* **1**. *Z. Anat. EntwGesch.* **90**, 412.
WANG, C. C. (1918). *J. Anat., Lond.*, **52**, 107.
WYBURN, G. M. (1937). *J. Anat., Lond.*, **71**, 201.
ZIEGLER, H. E. (1887). *Arch. mikr. Anat.* **30**, 596.

EXPLANATION OF PLATES 1–4

The photomicrographs in Pls. 2–4 have been reproduced without retouching.

PLATE 1

Fig. 1. A drawing of part of the wall of the yolk sac in the abembryonic region showing the different types of developing blood cells lying within, and in the neighbourhood of, a vascular space which is lined with endothelium. Section no. 31, the sections having been numbered from the anterior end of the embryonic disc. ×*c.* 750. Cell no.: 1, haemocytoblast; 2, extravascular haemocytoblast; 3, small haemocytoblast; 4, transitional haemocytoblast; 5, early primitive erythroblast; 6, intermediate primitive erythroblast (with indented nucleus); 7, binucleated primitive erythroblast; 8, intermediate primitive erythroblast; 9, late primitive erythroblast; 10, disintegrating erythroblast; 11, non-nucleated primitive erythrocyte; 12, binucleate giant cell; 13, remains of early blood cell.

Fig. 2. A drawing of part of the wall of the yolk sac near to its attachment to the embryonic disc. A well-formed blood island is seen in the right of the drawing. Cell no. 1 is a haemocytoblast and no. 9 a late primitive erythroblast. No. 14 shows the formation of an angiocyst in relation with the mesothelium. No. 15 shows a cell undergoing vacuolation (? transformation into an endothelial cell), the contents being discharged to contribute to embryonic plasma. A large vacuole is formed in one of the entodermal cells; other entodermal cells have 'ragged' edges suggesting that they have discharged their vacuoles into the yolk sac. Section no. 31. ×*c.* 750.

PLATE 2

Fig. 3. A section through the umbilical stalk in the region of the allanto-enteric diverticulum. Chromatic particles are conspicuous in many of the mesodermal cells, and are also present in some of the ectodermal and entodermal cells. Ventro-lateral to the allanto-enteric diverticulum is a group of primitive erythroblasts some of which are undergoing mitosis. Section no. 108. ×560.

Fig. 4. Section through the blastopore and beginning of the chorda canal. The anterior lip of the blastopore lies to the left of the photograph. The cells are columnar both in the superficial ectoderm and in the roof of the chorda canal. The nuclei are deeply stained and are, for the most part, situated deeply, near the basement membrane. The cytoplasm is clear and the cell boundaries are mostly well defined. Section no. 46. ×560.

PLATE 3

Fig. 5. A section of the definitive cloacal membrane. It shows the continuity of ectoderm and entoderm. The mesodermal cells pass around the side of the membrane. The amniotic cavity is situated above in the figure. Section no. 96. ×560.

Fig. 6. A section through the prochordal plate. The entodermal cells are seen to be cuboidal. Chromophilic granules are seen in some of the cells and between the plate and the ectoderm. Section no. 28. ×700.

Fig. 7. The section shows an empty capillary vessel, cut transversely, which is lying in the mesenchymal tissue of a chorionic villus; it is bounded on one side by angioblast which sends processes outward into the surrounding mesenchyme. An isolated angioblastic strand lies below the vessel, but does not appear to take part in the formation of its wall. Section no. 93. ×350.

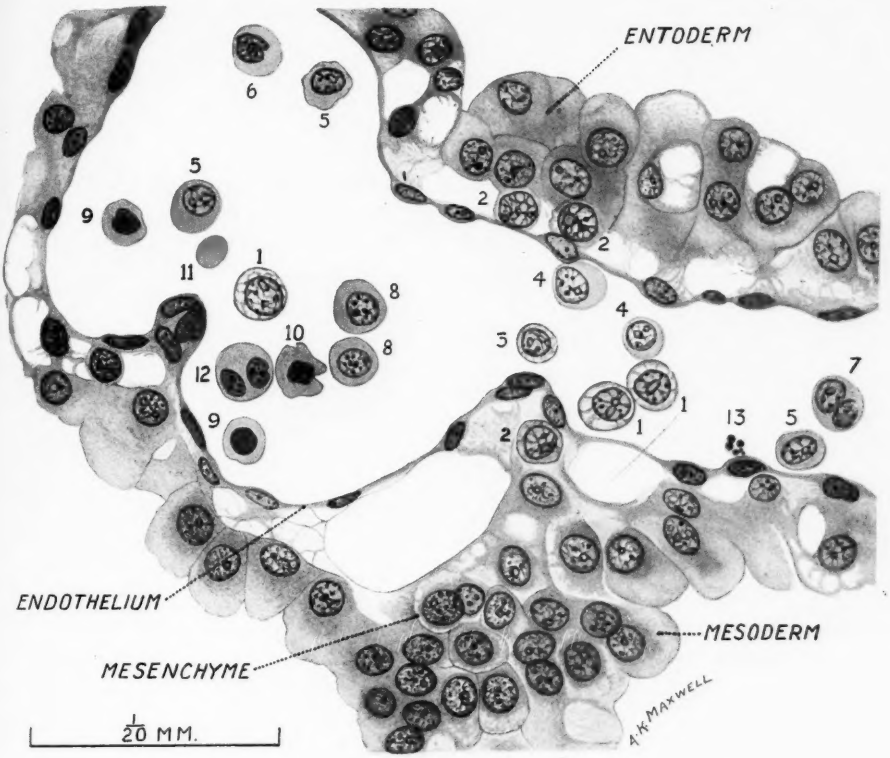
Fig. 8. In the upper part of the figure, maternal red blood corpuscles are seen in the intervillous space. Below this is the trophoblast which shows a distinct division into plasmodial and cytotrophoblastic layers. In the chorionic mesoderm angioblastic strands and an early stage in the development of capillary vessels may be seen. Section no. 30. ×350.

PLATE 4

Fig. 9. The section passes through the basal or chorionic end of the connecting stalk, where the supporting mesenchymal tissue resembles, and is continuous with, the parietal layer of the chorionic mesoderm. A well-developed vascular channel crosses the lower part of the photograph. The endothelial wall of this channel shows various stages in the 'rounding up' and liberation of endothelial cell elements, some of which are seen lying free in the lumen of the vessel. Note the contrast between these cells and those in Pl. 4, fig. 11. Note also extension of solid angioblastic strands into the mesenchyme on the left. The group of epithelial cells in the upper central part of the figure probably represent a degenerate remnant of the distal portion of the allanto-enteric diverticulum. Section no. 144. ×350.

Fig. 10. Section through a portion of the wall of the foregut which is shown at a lower magnification in Text-fig. 8. A small blood island is seen between the entoderm lining the floor of the foregut and the mesothelial layer covering it superficially. In the roof of the foregut opposite the blood island is a triangular space which can be traced through a series of sections. The base of the triangle is formed by entoderm; the inner and outer walls, which converge to the apex of the triangle, closely resemble the angioblastic tissue found in the chorionic mesenchyme and the basal segment of the connecting stalk. Section no. 10. ×560.

Fig. 11. Segment of wall of yolk sac showing entoderm on the right side and mesothelium on the left. In the upper part of the figure the entodermal cells show signs of degeneration; in the lower part the cells appear to have been shed, leaving the endothelial wall of a vascular space exposed. In the mesoblast is a blood island which is covered, in the greater part of its extent, by endothelium; it projects below into the vascular space and appears to be breaking through previous to discharging free erythroblasts into the lumen of the space. ×560.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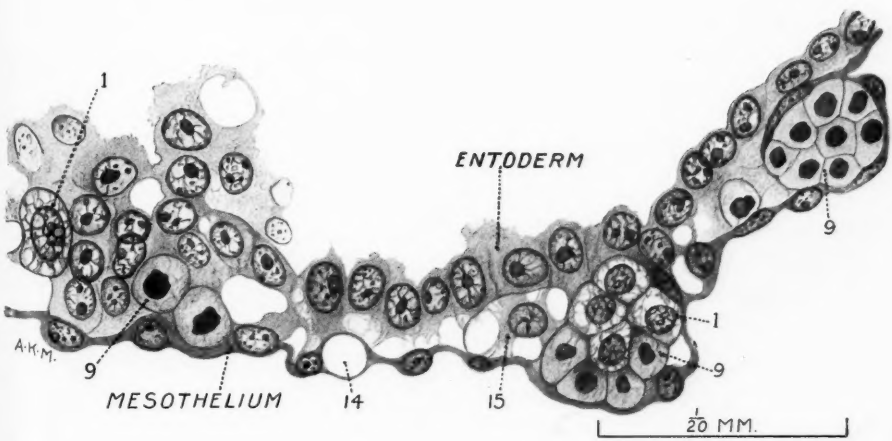
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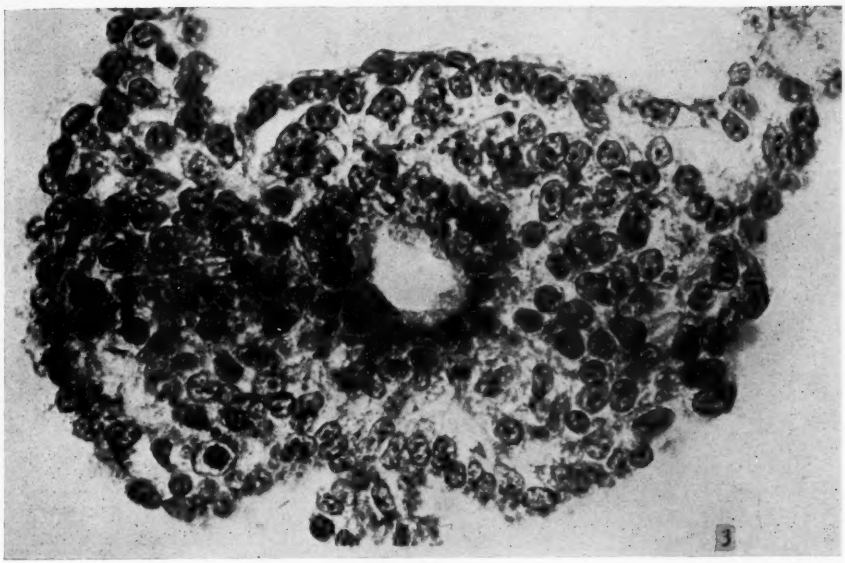

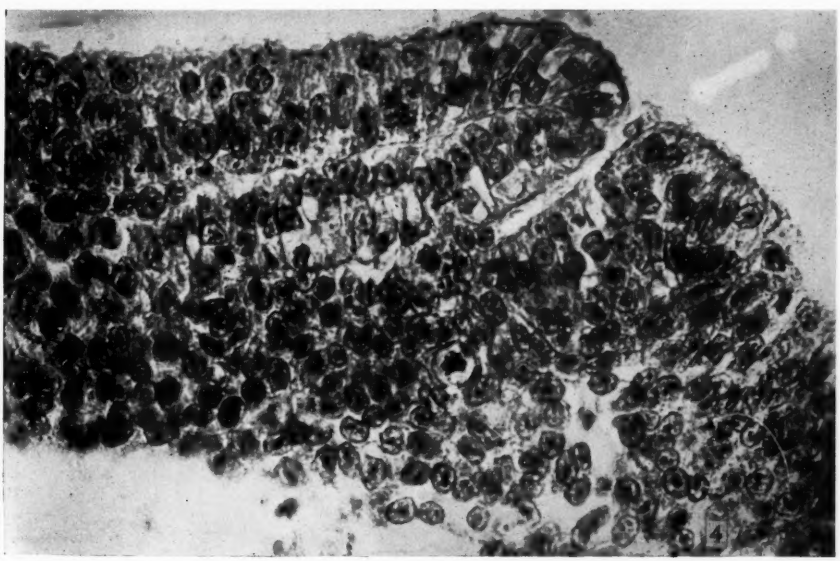

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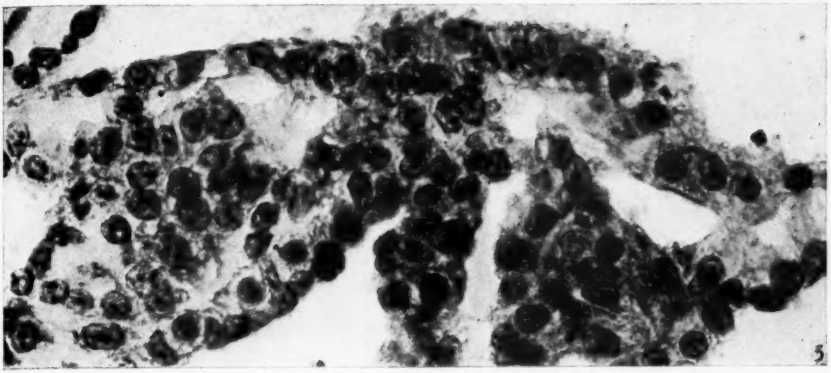

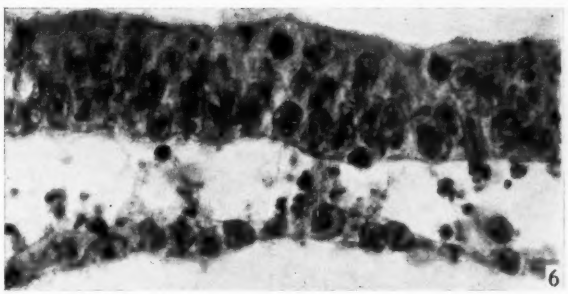

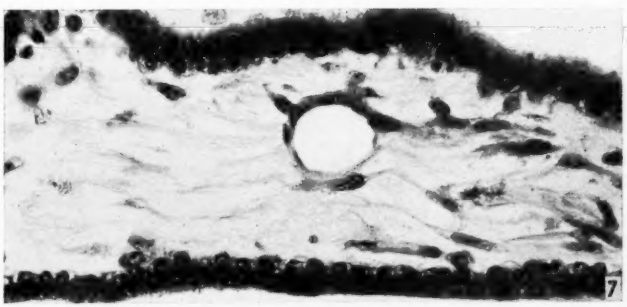

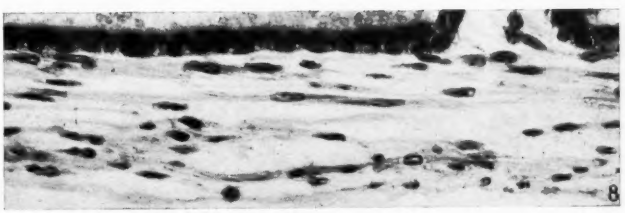

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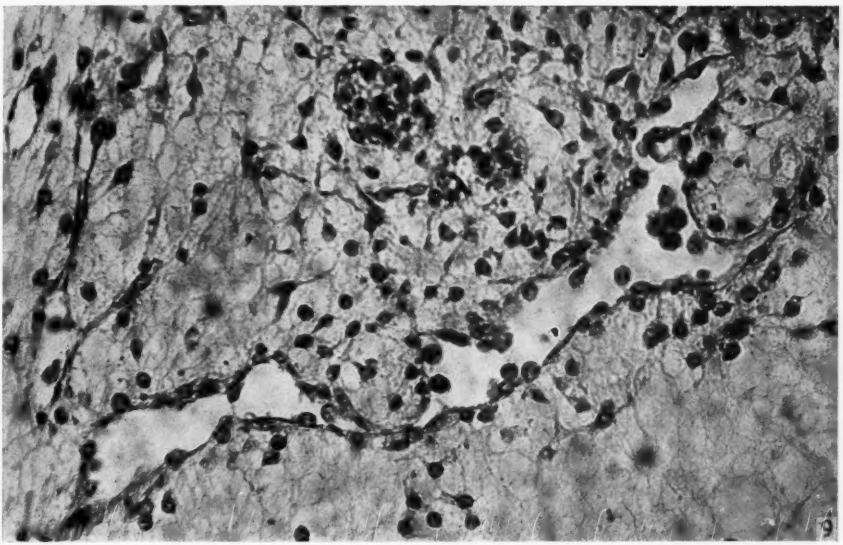

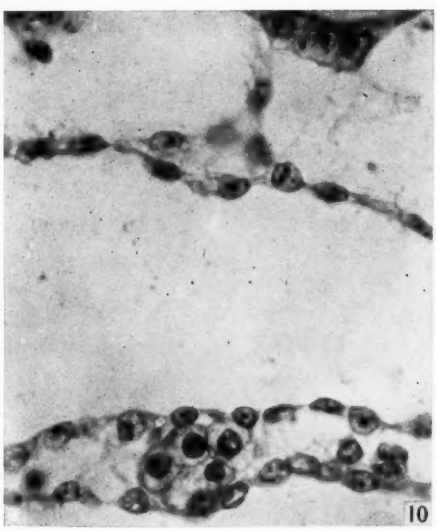

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A COMPARATIVE STUDY OF THE INFERIOR TIBIO-FIBULAR JOINT

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In the literature of comparative anatomy, it is not uncommon to find the assumption that the second section of the hindlimb has abandoned an ancestral mobility still exhibited in the forearm. References are made to this mobility having become 'limited' or 'restricted', and the arboreal metatheria are spoken of as having 'retained' a freedom of movement between the tibia and fibula, which has been 'abandoned' in other mammals. Though this assumption is by no means universal, it seems sufficiently widespread to justify an enquiry into the evolutionary history of the articulations and movements of the tibia and fibula, with a brief consideration of their mammalian variations.

About the origin of the cheiropterygium or tetrapod limb, nothing is known for certain. According to Goodrich (1930), 'it may have been derived from a fin not unlike that of the Devonian Dipnoi or Crossopterygii.... We should expect the fish-like ancestors of the Tetrapoda to have possessed pectoral and pelvic fins alike in structure.' The word 'alike' is liable to misinterpretation. That the limbs are similar in general pattern is obvious. But there is no proof that they were ever identical, or that any exact muscular homology ever existed between them. Indeed, it seems more probable that the tetrapod fore- and hindlimb evolved differently from their very beginning. Romer & Byrne (1932), in a fundamental article on the pes of *Diadectes*, have put forward evidence that the pectoral and pelvic fins in the fish ancestors of Tetrapoda were already differently orientated, and that the flexor aspect of the forelimb corresponds to the outer surface of the pectoral fin of such a form as *Ceratodus*, whereas the flexor aspect of the hindlimb corresponds to the inner surface of the pelvic fin. According to these authors, embryological work on fishes, as well as a study of the musculature of lizards, confirms the correctness of this interpretation. They believe further that, in consequence of the different position of the fin surfaces, the hand and foot[1] acquired their forwardly pointing position by different means, involving a complete twist of the forelimb on its long axis around the elbow joint, and a pivot or adduction movement of the hindlimb at the ankle joint, at the expense of its inner border. For the somewhat complicated explanation of this process, reference should be made to the original paper. Romer's view of limb development certainly accords with the morphological contrasts shown in the two limbs, such as the sharp change of plane between the humerus and forearm and the continuity

[1] The terms 'hand' and 'foot' are used for brevity to indicate the extremities of the fore- and hindlimbs.

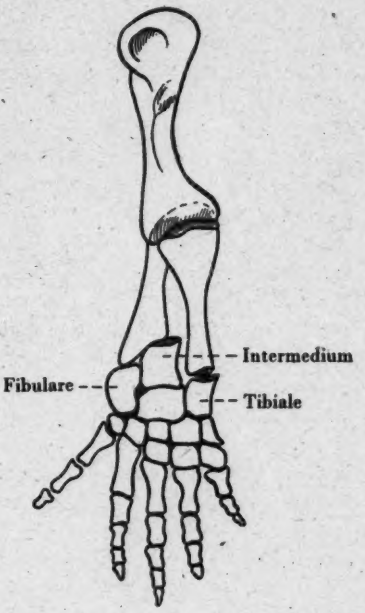
of plane between the thigh and leg, the primitive strength of the extensors in the case of the forearm and of the flexors in the case of the leg, the development of rotational movement in the forearm and its absence in the leg, and the retention of the radiale and the loss, fusion, or displacement of the tibiale.

Whether Romer's explanation of these evolutionary differences be accepted or not, it is noteworthy that they appear to have existed as far back as the phylogenetic history of vertebrates can be traced. Broom (1930), though he holds different views from Romer on the interpretation of fin-limb evolution, supports the idea of an early divergence between the two. He produces suggestive evidence that the conversion of a fin into a limb did not occur simultaneously in both extremities, but appeared first in the forelimb. It is now generally agreed that it is among the lobe-finned fishes that the tetrapod ancestor must be sought. One of these, *Sauripterus*, an American Upper Devonian fossil, has a fin of great interest. As Broom has pointed out, the front part is so formed as to suggest that it was used for scooping sand or mud, while the greater part of the fin was still employed as a swimming organ. From such a beginning, the anterior part might well develop into a crawling limb, while the hinder part would then gradually disappear. An incipient form of this condition exists in the modern gurnard, which exhibits crawling 'fingers' on the front of what is otherwise a swimming fin. It seems very likely that while the pelvic fin still functioned purely as a fin, a part of the pectoral fin was beginning to develop into a limb. The early amphibia, which probably emerged in the Devonian period with its seasonal droughts, were not so much concerned with getting on to the land, as with getting off the drying land into water again, and therefore it is hardly to be expected that both fins would have started simultaneously to abandon their original function. This, as Broom says, would have resulted in countless generations having neither a good fin nor a good limb.

It is generally recognized now that the primitive amphibians advanced over the land, as they continued to do through the water, by sinuous movements of the trunk which brought forward first one flat limb and then the other, very much as the salamander moves to-day, but, as the sandy surface gave way to vegetation, limb flexures came into being to permit the animal to be lifted off the ground and the joints began to acquire freedom. Whereas joints in fish are synarthroses, or, at the most, partially split synarthroses, diarthroses now begin to be found. A consideration of recent primitive vertebrates suggests that, in a phylogenetic series, the first free joints to appear in Amphibia are the shoulder, elbow and hip (Lubosch, 1938). This adds another item to the list of primitive differences between the fore- and hindlimbs and accords with the early appearance of rotational movements in the forearm. The sum total of slight movements between the mass of cartilaginous elements presumably gave sufficient freedom to the hinder extremity at this stage. The knee-joint in Amphibia is only partly free, and in the urodeles, according to

Lubosch, it resembles a digital hinge joint, with a cartilaginous cushion in front and synovial protrusions behind, but no menisci.

At this stage of evolutionary history, it is possible to abandon conjecture for the study of primitive living forms or of fossil forms which, if not directly ancestral to higher vertebrates, were at least not far removed from the main line. It would appear that, with the first angulation that produced a definite limb, the necessity of turning the foot forwards must have thrown the weight on the inner bone or tibia, and thus from the start (at least in the case of an angulated limb), the tibia articulated with both condyles of the femur, and the fibula with the side of the lateral condyle. This is well seen in *Trematops* (Text-fig. 1), generally considered a most primitive amphibian type, and would seem to preclude any greater degree of rotation than the slight twist still exemplified in man. In other fossil Amphibia, such as the labyrinthodont *Uranocentrodon*, or the newt-like *Scincosaurus*, though the distal tarsal elements vary considerably, the proximal row of tibiale, intermedium and fibulare (and their relations to the leg bones) remain alike in all; the tibia articulates with tibiale and intermedium and the fibula with fibulare and intermedium, but the facets on the intermedium for tibia and fibula are separated by a notch, and the two bones are not in contact at their lower ends.

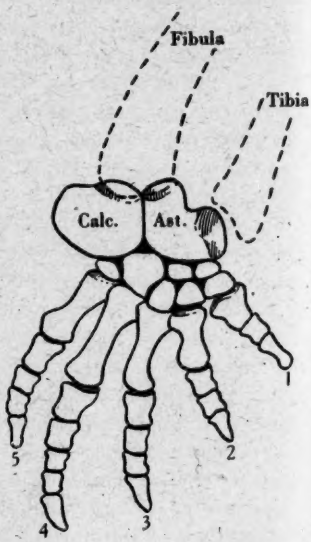


Text-fig. 1. Hindlimb of *Trematops*, a primitive Permo-carboniferous amphibian (after Case *et al.* 1913).

The musculature of these extinct animals is obviously a matter of conjecture, but the arrangement may be partially inferred from a study of the salamander. Though the latter is a urodele amphibian, and therefore a specialized descendant of the common vertebrate stock from which mammals are derived, the articulation of the leg with the proximal tarsal elements is so like the primitive fossil amphibian arrangement, that a study of the musculature of this region is of particular interest. The muscle masses in the salamander are only very partially differentiated, and it is a difficult problem to define them sharply, hence the confusing variations in description and nomenclature in the different accounts.

As this study is concerned chiefly with rotational movements between the tibia and fibula, or eversion and inversion of the foot, it is sufficient to note

only the muscles concerned in such movements. 'Pronation' in the hindlimb of the salamander is produced by three muscles (Francis, 1934): (1) *flexor accessorius lateralis*, passing from the distal end of the fibula and the lateral edge of the tarsus to the plantar fascia near the middle; (2) *flexor accessorius medialis*, a larger muscle, arising from the distal two-thirds of the fibula and the lateral part of the tarsus and becoming attached to the plantar fascia medial and distal to the preceding muscle; (3) *pronator profundus*, the deepest of the three, from the medial side of the fibula, deep to the *flexor accessorius medialis*; its fibres converge to an insertion into the lateral face of the distal end of the tibia, the tibiale, the cartilago pre-hallucis and the base of the first metatarsal. Eisler calls this muscle 'fibulo-tarsalis'. Francis gives the action of the first two as 'accessory to the flexor primordialis communis, with a distinct pronating action'. It will be seen that of the three pronating muscles, two act entirely and one chiefly on the ankle and intertarsal joints. On the other hand, it appears that the range of rotational movement of the tibia itself cannot be any greater than the slight screw of higher forms. That part of the pronator which is inserted into the tibia is attached to the lateral aspect, and not, as one would expect of a true rotator, across the bone to its medial border. The only muscle producing the contrary movement of inverse rotation or 'supination' in the salamander is the '*extensor tarsi tibialis*' ('rotateur inverse du pied' of Perrin), a small muscle passing from the lateral femoral epicondyle to the ventral surface of the tibiale and cartilago pre-hallucis.



Text-fig. 2. Foot of *Ophiacodon*, a primitive Permo-carboniferous reptile (after Case *et al.* 1913).

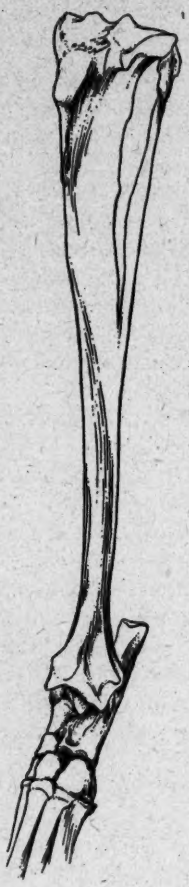
In reptiles, free diarthrodial joints appear and menisci develop in the knee joint. *Ophiacodon* (Text-fig. 2) is a primitive reptile roughly contemporary with *Trematops*. It has been described by Case *et al.* (1913) and the tarsus is held by them to be the most generalized known among reptiles. The proximal row consists of astragalus and calcaneum side by side, and there are two centralia and five tarsalia. The fibula articulates with the calcaneum and astragalus, the tibia with the astragalus only, and there is wide separation between the two articulations. At its upper end, the tibia has an oval surface for the lateral condyle of the femur and a crescentic surface for the medial condyle. The surfaces allow of forward and inward or of backward and outward movement. The fibula articulates with the side of the lateral condyle and has the same range of movement. It is interesting to note the difference in the

elbow joint, in which a large circular capitellum, comprising one-third of a sphere, articulates with a concavity on the radius. Here therefore, in a Permo-carboniferous reptile, we find already established the anatomical basis for movements which still characterize the second segments of the fore- and hind-limbs in man.

The muscles producing rotational movements in reptiles do not differ in essentials from those in amphibia. There is a well-developed pronator mass in both reptiles and monotremes, which is roughly divisible into three potentially or actually separate parts: (1) from the lower part of the fibula to the medial tarsals or the fascia in this region, (2) from the proximal part of the fibula to the astragalus, and (3) from the upper end of the fibula to the shaft of the tibia; the latter is the 'popliteus' element, whose origin migrates upwards in placental mammals to reach the femur. The inverse movements are produced, as in urodeles, by an extensor tarsi tibialis, but this has now descended from its femoral origin to the tibia and is found throughout reptiles, monotremes, marsupials and placental mammals as a conservative muscle represented in man by the tibialis anterior. There is, both in amphibia and in numerous reptiles (*Uromastix*, *Lacerta*, *Varanus*, *Gongylus*) an interosseous muscle deep to the pronator between tibia and fibula. According to Perrin (1892), however, both this and the 'popliteus' element of the pronator mass act only in preventing separation of the two bones. This arrangement would appear necessary, since the muscles passing from the thigh to the leg are inserted almost entirely into the tibia, whereas those passing from the leg to the foot arise nearly exclusively from the fibula. The two bones are separated at their lower ends, so that muscle action might drive them apart if it were not for a direct muscular connexion between them. In both amphibia and reptiles, there are peroneal muscles passing on the extensor aspect from the femur or fibula to the fibular side of the tarsus, but they appear to function as extensors only. It may therefore be seen that in reptiles, as in amphibia, rotational action of the lower part of the hindlimb is produced almost entirely by muscles acting on the tarsal joints.

When we pass from this simple arrangement to a consideration of the same region in mammals, a great diversity of form is found. Developmental trends appear to lead in various directions according to the uses to which the limb is put. A progressively greater degree of approximation of the lower ends, leading to partial fusion, is the evolutionary path followed by jumping animals. This fusion is seen in *Tarsius* (Text-fig. 3) and many of the Insectivora, and in jumping rodents such as the hare and rabbit (Text-fig. 4). The tenrec, an insectivore from Madagascar, shows a fusion of the tibia and fibula over the area generally occupied by the inferior interosseous ligament. When the limb is habitually used in action against a resistant medium such as earth or water (e.g. in burrowing or swimming), a powerful bony base is necessary for the muscles acting on the paws or flippers. This is obtained by a widely spread, strongly built tibia and fibula, strengthened by fusion at one or both ends, as

is well seen in *Priodontes*, the giant armadillo (Text-fig. 5). Fusion of the lower half or more of the bones is found in *Talpa* (Text-fig. 6), the European mole, and *Bathyergus*, the South African sand mole (Text-fig. 7).



Text-fig. 4.

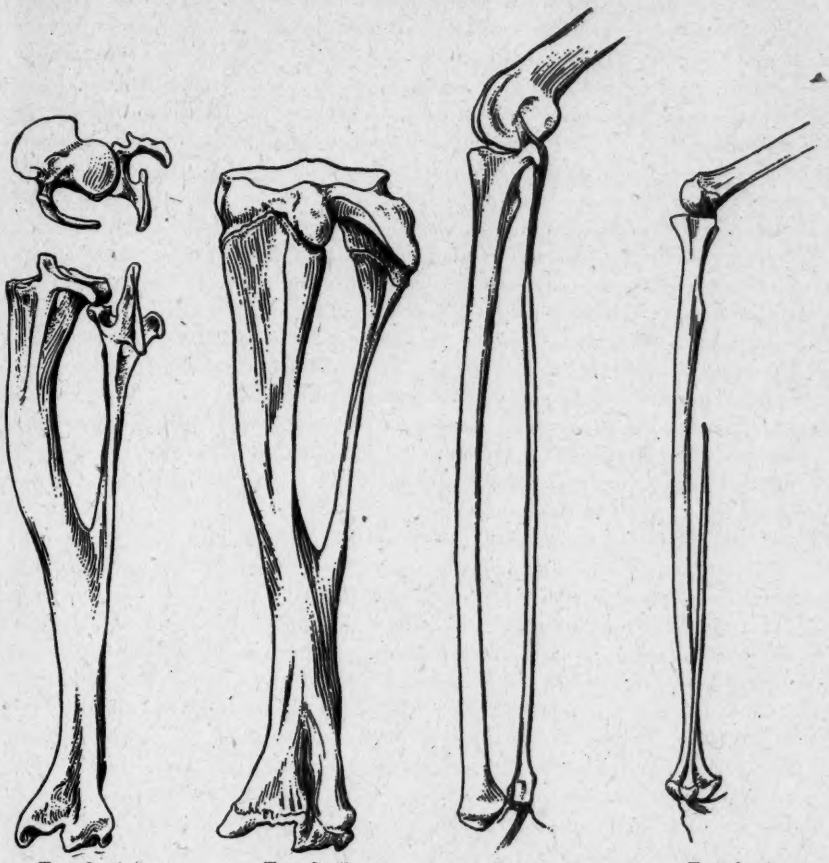
Text-fig. 3.

Text-fig. 5.

Text-fig. 3. Conjoined tibia and fibula of *Tarsius spectrum* (× 1).
Text-fig. 4. Conjoined tibia and fibula of *Lepus timidus* (× $\frac{1}{2}$).
Text-fig. 5. Conjoined tibia and fibula of *Priodontes giganteus*, the giant armadillo (× $\frac{1}{3}$).

Bats differ from all other vertebrates in having the hind-limb so rotated outwards by the wing membrane, that the knee is directed backwards like the elbow. When the animal rests on the ground, the foot would therefore point in the wrong direction, and this is corrected by a forward and medial rotation.

According to Flower and Lydekker (1891), this movement takes place at the ankle. The wing membrane in most species is attached to an elongated cartilaginous process, the calcar, and when this is so the fibula degenerates at the



Text-fig. 6. Text-fig. 7. Text-fig. 8. Text-fig. 9.

Text-fig. 6. Conjoined tibia and fibula of *Talpa europea* (× 4). Above, upper articular surface viewed from above; below, front view.
Text-fig. 7. Conjoined tibia and fibula of *Bathyergus maritimus* (× 2).
Text-fig. 8. Tibia and reduced fibula of *Vespertilio noctula* (× 6).
Text-fig. 9. Tibia and reduced fibula of *Pteropus edulis*, the flying fox (× 1).

upper end, where it is replaced by a cartilaginous or ligamentous strip. It may be reduced to a small process above the heel, as in *Megaderma*, or disappear altogether, as in *Nycteris*. The close resemblance between different species is illustrated in Text-figs. 8 and 9, which show the hindlimb of a common English

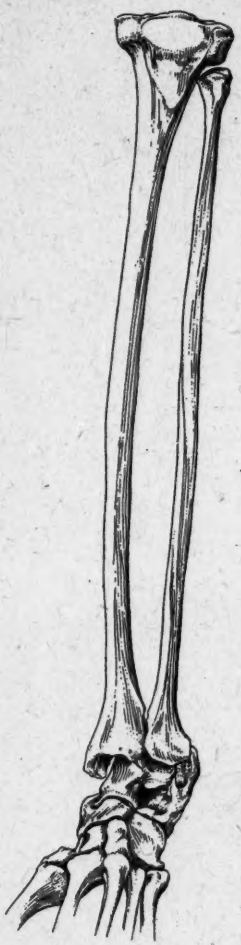
bat and that of the 'flying fox' of Malaya. In the Molossinae, however, the wing membrane is not attached to the foot and the fibula is well developed. This subfamily is credited by Flower and Lydekker with great dexterity in flight.

It is in carnivora and arboreal mammals that we find for the first time an articular facet between the lower ends of the tibia and fibula. Life in the trees demands agility and freedom of movement, and a carnivore likewise needs to be speedy and supple to grapple with its prey. It cannot afford, as Romer says, to develop the rigid skeleton or hoofed condition of the herbivora. Hence the appearance in both these groups of a diarthrodial joint extending upwards from the ankle joint. In its simplest form, this is no more than a slight smoothing or lipping of the lower ends of the bones. This was the condition in the only specimen of *Ptilocercus* that I was able to examine. It is also exemplified in the baboon. But in most carnivora and arboreal mammals, there is on the tibia a semilune with the base downwards, situated nearer the front than the back of the bone, and continuous inferiorly with the facet for the astragalus. It articulates with a similar facet on the fibula. These articular surfaces are strikingly similar in outline, form and position in all animals in which such a joint cavity exists. They are to be found in *Tupaia*, and in the arboreal sloths. They are well developed in the lemurs, and best of all in *Daubentonia*. They are present in *Macaca*, the gibbon (Text-fig. 10), the chimpanzee (Text-fig. 11) and the gorilla. In man a slight lipping is to be found in 80 % of individuals, in the other 20 % there is a small facet.

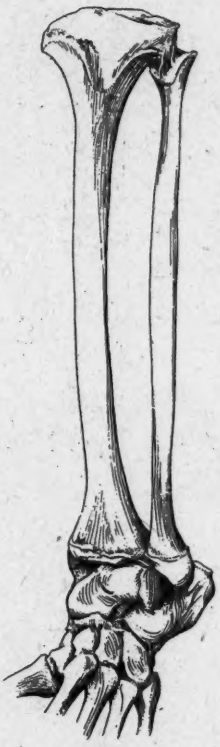
It is interesting to note that once again similar evolutionary paths have been followed by the marsupial equivalents of the various eutherian types. The kangaroos form a parallel to the jumping eutherian mammals, and in *Macropus* (Text-fig. 12), though there is not actual fusion, the tibia and fibula are so moulded together in their lower halves as to form what appears to be one cylindrical bone with a crack in it. *Thylacomus*, the common rabbit bandicoot, shows the same fusion of the lower half of the leg bones as has already been observed in Rodentia (Text-fig. 13). The mobility of the ankle joint in Carnivora, as indicated by the presence of an inferior tibio-fibular facet, is also to be seen in *Thylacinus*, the Tasmanian wolf. *Meles*, the badger (Text-fig. 14), has an ankle joint very similar to *Sarcophilus*, the Tasmanian devil (Text-fig. 15). Finally, the phalangers show a striking resemblance to the arboreal placental mammals in the presence of semilunar facet, while *Dasycercus*, a primitive marsupial, exhibits the small synovial extension from the ankle joint upwards seen in *Ptilocercus*, and most commonly in man.

Embryonic development. It was thought that light might be thrown on the evolutionary history of the inferior tibio-fibular joint by the examination of this region in human embryos. Accordingly the foot and ankle region of embryos of 90, 130, 150, 220 and 330 mm. was decalcified, cut in serial sections and stained with Weigert's haematoxylin and van Gieson. At the 90 mm. stage there is as yet no joint cavity formation in the ankle region, but the general relationship of tibia and fibula is much the same as in later life (Pl. 1*a*).

At the 150 mm. stage, spaces have appeared round the tendons of the peroneal and flexor muscles, and to a slight extent along the line of the future ankle joint. The 220 mm. stage (Pl. 1*b*) shows a well-formed joint cavity with



Text-fig. 10.



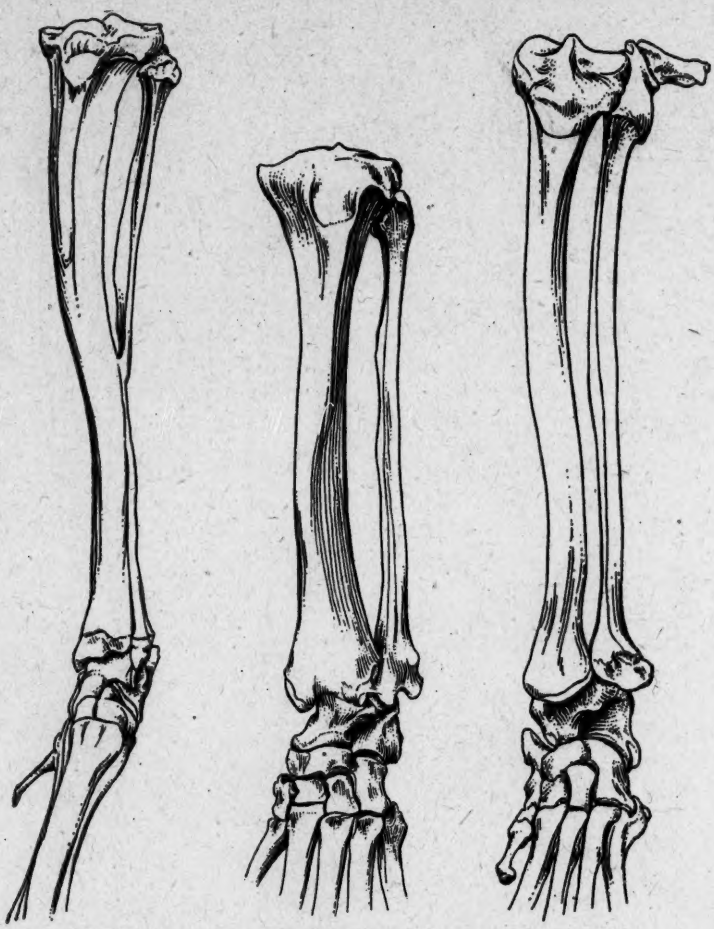
Text-fig. 11.

Text-fig. 12.

Text-fig. 10. The bones of the leg and ankle of *Hylobates lar* ($\times \frac{2}{3}$).
Text-fig. 11. The bones of the leg and ankle of *Pan satyrus* ($\times \frac{2}{3}$).
Text-fig. 12. The bones of the leg and ankle of *Macropus* ($\times \frac{1}{4}$). Cf. Text-figs. 3, 4.

mesenchymal discs projecting into it on both sides. According to Santo (1931), these mesenchymal discs are also found in pig, rabbit and guinea-pig embryos. The frequency of such intra-articular discs in embryos suggests that these

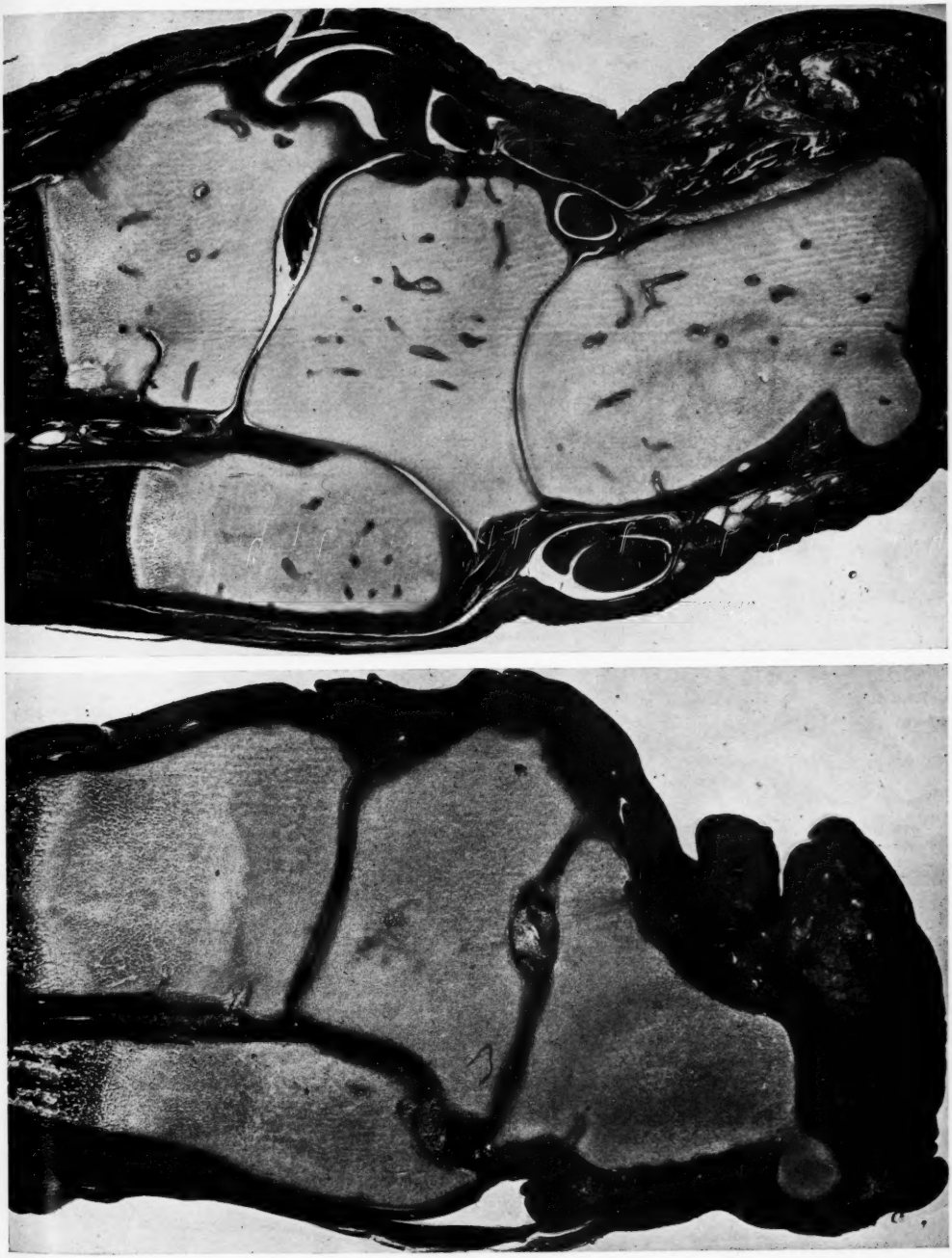
structures are part of a primitive joint mechanism, still preserved in some joints, but lost in the majority for reasons which are still obscure. This interpretation receives some support from a study of the primitive marsupial



Text-fig. 13. Text-fig. 14. Text-fig. 15.

Text-fig. 13. The bones of the leg and ankle of *Thylacomus lagotis* (× 1). Cf. Text-fig. 4.
Text-fig. 14. The bones of the leg and ankle of *Meles* (× ½).
Text-fig. 15. The bones of the leg and ankle of *Sarcophilus ursinus* (× 1).

Dasycercus. In this animal, a disc is seen projecting from the fibula between the tibia and the talus, in the manner of the 220 mm. human embryo, and another exists between the navicular and the first and second cuneiforms.



(*b*) 220 mm. human embryo. Same. × 8.

(*a*) 90 mm. human embryo. Coronal section through lower end of leg and ankle. × 17.

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In brief, the palaeontological, comparative anatomical and embryological evidence suggests that at no time has there been any greater power of rotational movement of the tibia on the femur or fibula than at present exists in man. The Carnivora and the arboreal mammals have not 'retained' but have developed an inferior diarthrodial tibio-fibular joint, and the latter could not have existed in primitive types because the two bones were not in contact. Rotational movement of the tibia could, of course, take place without such a joint, but nothing in the shape of the knee or ankle-joint, or in the disposition of muscles in primitive types, suggests that such movement did, in fact, occur. Nor does ontogenetic development give any support to the theory that there was at any time, in the evolution of man, greater mobility between the two bones.

SUMMARY

1. The early evolution of the hindlimb is briefly reviewed, with reference to fossil forms already described. The rotational muscles in primitive amphibia and reptiles are discussed. The bones of the leg and ankle region have been examined in a number of mammalian forms, and fresh specimens of this region in *Ptilocercus* and *Dasycercus* have been dissected.

2. Serial sections of the inferior tibio-fibular and ankle region have been made in human embryos of 90, 130, 150, 220 and 330 mm. C.R. length.

3. The palaeontological, comparative anatomical and embryological evidence suggests that at no time has there been any greater power of rotational movement of the tibia on the femur or fibula than at present exists in man. A diarthrodial inferior tibio-fibular joint appears for the first time in carnivorous and arboreal mammals.

The author wishes to thank Prof. Le Gros Clark for his helpful comments and suggestions, Prof. Wood Jones for the specimens of *Dasycercus*, Miss Elizabeth Frazer for the line drawings (except Text-figs. 1, 2), and Mr W. Chesterman for preparing the serial sections of human embryos.

REFERENCES

Broom, R. (1930). *The Origin of the Human Skeleton.* Edinburgh: Riverside Press.

Case, E. C., Williston, S. W. & Mehl, M. G. (1913). 'Permo-carboniferous vertebrates from New Mexico.' *Publ. Carneg. Instn.*

Eisler, P. (1912). *Muskeln d. Stammes.* Jena.

Flower, W. H. & Lydekker, R. (1891). *Mammalia—Living and Extinct.* London: A. and C. Black.

Francis, E. T. B. (1934). *The Anatomy of the Salamander.* Oxford: Clarendon Press.

Goodrich, E. S. (1930). *The Structure and Development of Vertebrates.* Macmillan and Co.

Lubosch, W. (1938). *Handb. vergl. Anat.* 5, sect. IV.

Perrin, A. (1892). *Bull. Sci. Fr. Belg.* 24, 372.

Romer, A. S. & Byrne, F. (1932). *Palaeobiologica,* 4.

Santo, E. (1931). *Z. ges. Anat.* 1. *Z. Anat. EntwGesch.* 104, 623.

THE CAPILLARY BED OF THE CENTRAL NERVOUS SYSTEM IN A MEMBER OF A SECOND GENUS OF GYMNOPHIONA—*SIPHONOPS*

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That there are two remarkably distinct types of capillary bed in the central nervous system, consisting respectively of independent capillary loops in one case and of a continuous reticulum in the other, is now recognized. It is also established that the latter type occurs in amphibians of the order Salientia, and the former in Caudata (Schöbl, 1882; Sterzi, 1904; Craigie, 1938*a*, 1938*c*). The interesting problem of the phylogenetic relationship between these two types of capillary bed, and the somewhat crucial phylogenetic position which many authorities believe the Gymnophiona to occupy, lend special interest to the examination of the mechanism in question in that relatively unfamiliar order. This matter was discussed in a recent paper (Craigie, 1940*b*) in which it was reported that *Dermophis mexicanus* has a central nervous system supplied with capillaries entirely of the simple, independent loop type.

Although the observations on *Dermophis* make it appear probable that the simple capillary loops are the arrangement present in the brains of Gymnophiona generally, it is highly desirable to have confirmatory evidence from other genera. Hence a note is offered on conditions in *Siphonops*, of which material was obtained just after the paper on *Dermophis* had been submitted for publication.

MATERIAL

The material used consists of five brains of *Siphonops annulatus* (Mikan) collected, prepared and very generously presented by Dr P. Sawaya, of Sao Paulo, Brazil, to whom the writer's deep gratitude is due. Three of the specimens were preserved by vascular injection with formalin, followed by immersion; the other two had the vessels injected with coloured gelatin and were fixed by immersion in formalin. Unfortunately, the gelatin reached only a few of the cerebral vessels in only one of the specimens. Excellent demonstration of the capillaries was secured, however, by staining with reduced silver, with Weil's myelin stain, and with Mallory's phosphotungstic-acid-haematoxylin. Serial sections were cut (three series transverse, one sagittal, one coronal), one series 20μ thick, three 30μ and one 100μ thick. In the case of three specimens, the whole head was decalcified and sectioned. Celloidin was used for embedding.

The brain of *S. annulatus* has been described and illustrated by Kuhlenbeck in his monograph on the brain of Gymnophiona (1922).

OBSERVATIONS

Angioarchitecture. The observations on the morphology of the capillaries in ***Siphonops*** are in full agreement with those in ***Dermophis*** already reported (Craigie, 1940*b*). All parts of the brain and the upper portion of the spinal cord are vascularized exclusively by long, slender, independent capillary loops arranged mainly in an approximately radiating manner, more or less perpendicular to the surface through which they penetrate, though sometimes curving to run some distance in other directions before terminating (Fig. 1). A photomicrograph of a single isolated loop is reproduced in Fig. 2. The two limbs of each loop are in close contact, are never branched, and appear to lie in a single perivascular space. Here and there, but not often, a secondary loop is formed by one limb of a primary loop (Fig. 3). As in the previous cases reported, such loops are found only in the substance of the central nervous organs.

These capillary loops seem to be in all respects similar to those occurring in Caudata. The tendency to spiral twisting of a loop about its own axis (Fig. 4) is somewhat less marked than in many of the latter. While a loop may lie in any plane, one limb is frequently in front of the other, so that longitudinal sections are particularly favourable for this study.

The use of preparations in which the capillary wall is stained instead of the vessels being either injected with an artificial mass or filled with stained blood corpuscles emphasizes more than does the latter type of material the closeness of contact between the two limbs of the single capillary loop. This is shown in the photograph published as Fig. 1 of the paper on ***Dermophis*** and in Fig. 6 of Scharrer's (1939) paper on the opossum. A careful search revealed occasional points at which the two capillaries appeared to be separated by a slight cleft, but almost everywhere there was direct contact, usually so close that the two opposed walls were flattened. A series of sections of a ***Necturus*** brain which was prepared by the phosphotungstic-acid-haematoxylin method for comparison confirmed the fact that the association is similarly close in that animal.

The functional value of the close contact referred to was discussed in a former note (1938*b*) and by Scharrer (1939).

The use of serial sections of the whole head made it possible to check the absence of capillary loops in tissues of that region other than the central nervous organs and the observations fully confirmed this. Even the peripheral nerves are vascularized by reticular vessels not running in pairs, the change of arrangement taking place precisely at the surface of the brain.

The condition of the vessels in the small pars nervosa of the hypophysis is not clear in the sections, but it is probably an infolding of the meningeal vascular net, as in Caudata (1938*d*). The vascular bed of the pars distalis of the hypophysis is a spongy reticulum.

The paraphysis (like that of ***Amblystoma*** described by Herrick (1935) and by Roofe (1935)) also contains a rich spongy reticulum of wide sinusoids (Fig. 6). Conditions in the small epiphysis are somewhat obscured by a

Fig. 1. Transverse section of right cerebral hemisphere of *Siphonops annulatus* stained with phosphotungstic-acid-haematoxylin. Thickness 100 μ. $\times$50.

Fig. 2. Photomicrograph of a single short capillary loop of *Siphonops* which has been completely withdrawn from the brain (dorsolateral pallium) by shrinkage. Weil's myelin stain. ×600.

Fig. 3. Photomicrograph of part of a loop in the white matter of the primordium hippocampi showing the formation of a secondary loop without branching of the capillaries. Weil's myelin stain. Thickness 30 μ. ×600.

tendency to over-staining, but no vessel was detected within the organ, which seems to depend for its blood supply entirely on the pial vascular net.

Calibre of the capillaries and size of the erythrocytes. The calibre of the two component capillaries was measured in twenty-five loops in the medial longitudinal bundle and the same number in the hippocampal region of four

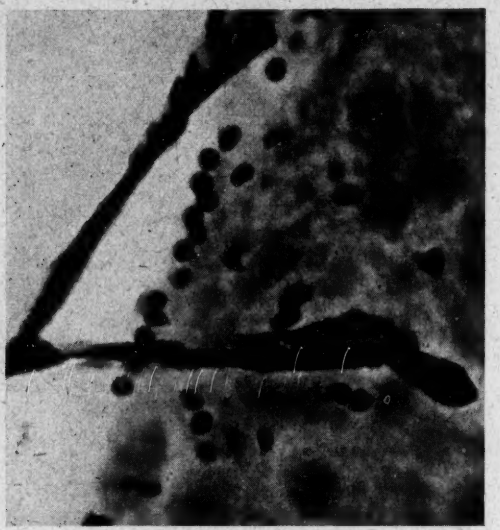

Fig. 4. Photomicrograph of a somewhat twisted loop in the accessory olfactory bulb. Coronal section 30 μ thick. Weil's stain. ×400.

Table 1. *Diameter in micra of capillaries (average of twenty-five loops) in brain of* Siphonops*—not corrected for shrinkage*

	Primordium hippocampi			Medial longitudinal bundle			
Siphonops	Thicker limb	Thinner limb	Average of both	Thicker limb	Thinner limb	Average of both	All capillaries measured
1	—	—	—	12·2	9·8	11·0	—
2	11·2	9·2	10·2	7·7	9·0	8·4	—
3	14·4	11·9	13·2	—	—	—	—
4	17·3	15·1	16·2	15·1	13·3	14·2	—
6	14·0	10·9	12·4	12·0	10·4	11·2	—
Average	14·2	11·8	13·0	12·1	10·1	11·2	12·1

specimens, with the results shown in Table 1. The values obtained are considerably larger than the corresponding figures for *Dermophis* (Craigie, 1940*b*), where the average diameter of the capillaries in the same two regions (likewise not corrected for shrinkage) was found to be 9·6μ. In both caecilians the difference in the calibre of the vessels in the two regions considered is greater

than in most animals where such measurements have been made, the hippocampus having markedly wider capillaries than the medial longitudinal bundle.

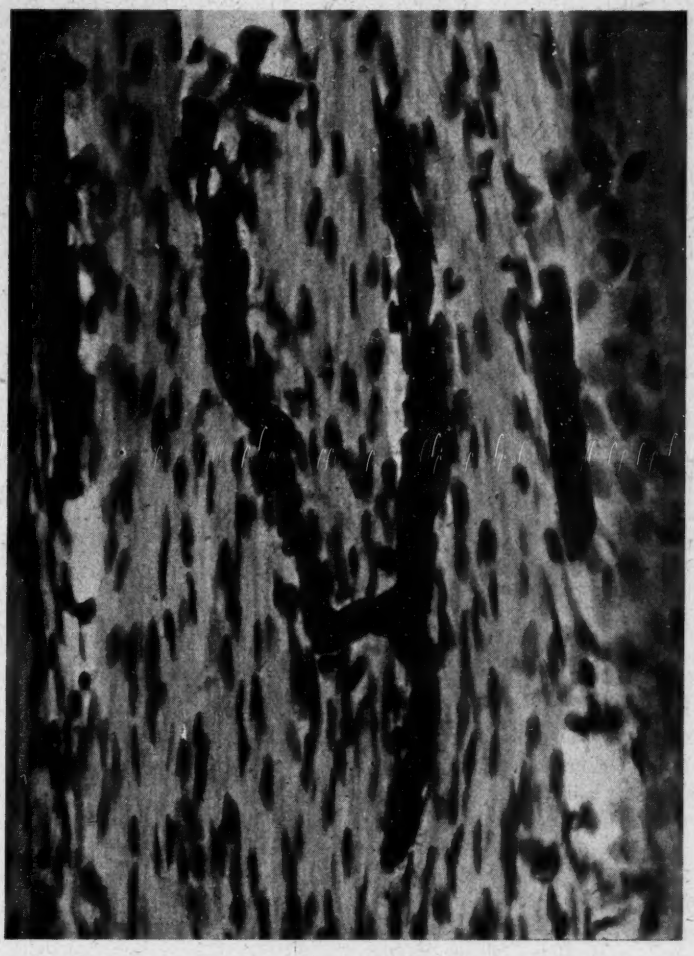

Fig. 5. Photomicrograph of part of the olfactory nerve in a coronal section 30 μ thick. The blood-filled capillaries are jet black after Weil's stain. $\times 300$.

Measurement of a number of erythrocytes, where they were not crowded but lay free, in the three specimens of which the whole head was sectioned, yielded a value of about $35 \times 22 \times 3{\cdot}6\mu$ for the size of these cells, which shows

the two larger dimensions to be considerably greater than in *Dermophis* (Craigie, 1940*b*) where similar uncorrected measurements indicated the corpuscles to be about $22 \times 15 \times 3{\cdot}8\mu$.

If the shrinkage of 17% in the two larger dimensions assumed for the red blood corpuscles of *Dermophis* be applied here also, these dimensions for the fresh cell are found to have been probably in the neighbourhood of $42 \times 26{\cdot}5$, which is about the size of the corresponding cells in *Cryptobranchus* (Gulliver, 1875; Smith, 1925). Hence *Siphonops* may be associated with the small group of tailed amphibians which have the largest erythrocytes known.

Fig. 6. Photomicrograph of small parts of the paraphysis and of the adjacent pallial white matter showing the vascular reticulum in the former and isolated capillary loops in the latter Saggital section 30μ thick, stained by Weil's method. ×100.

Thus the larger calibre of the capillaries in *Siphonops* as compared with those in *Dermophis* is undoubtedly significant, though it is not proportional to the dimensions of the red blood cells. It would appear that the capillaries are of greater calibre than those of *Cryptobranchus* (Craigie, 1938*a*), though the difference is not so great as comparison of the published values would suggest, since the measurements for *Cryptobranchus* were made on gelatin-injected vessels. For the same reason, the apparently greater calibre in *Siphonops* indicated by comparison with the values for *Necturus* (Craigie, 1940*a*) is deceptive.[1]

[1] Measurements of the capillaries in the primordium hippocampi of one specimen of *Necturus* stained with phosphotungstic-acid-haematoxylin yielded values of 14·4 and $11{\cdot}8\mu$ for the thicker and thinner limbs, or $13{\cdot}2\mu$ for all the capillaries. In a second they averaged $14{\cdot}5\mu$. In another specimen, which was gorged with blood (stained with Weil's myelin stain), the corresponding values were 24·4, 21·3 and $22{\cdot}8\mu$ respectively. It is evident that the apparent calibre of the capillaries, while experience has shown it not to be directly related to the pressure used in preparing injected material (Craigie, 1938*c*), is nevertheless largely influenced by the contents of the vessels when fixed.

Richness of vascularization. In order that a rough idea of the richness of the vascularization might be obtained, measurements of the total length of the capillaries in a unit volume of tissue were made in eleven regions of four specimens (the fifth series proving unsatisfactory for this purpose). The results were corrected for shrinkage by application of the coefficient found to be required for this purpose in *Necturus*, namely, 0·62 (Craigie, 1940*a*), direct measurement of shrinkage not having been possible. The corrected averages are presented in Table 2.

While these results can be regarded only as, at most, rough approximations, they show that all parts of the hindbrain studied are more richly vascularized than the corresponding parts in *Necturus* but considerably less so than those in *Amblystoma tigrinum* (1939). Only the cochlear nucleus has a supply equal to that of the eighth nerve nucleus in the latter animal.

Table 2. *Total length in micra of the capillaries in* 100^3 *cu.μ of fresh tissue in the brain of* Siphonops annulatus, *average of four specimens*

(Coefficient applied to correct for shrinkage 0·62)

Fasciculus long. medialis	51	Primordium hippocampi	67
Nucleus XII	46	Dorsal pallium (cells)	6
Nucleus motorius X	62	Dorsal pallium (white)	78
Nucleus fasciculi solitarii	50	Lateral pallium (cells)	9
Nucleus vestibularis	143	Lateral pallium (white)	82
Nucleus cochlearis	183		

In the forebrain, despite the more highly differentiated condition of the primordium hippocampi according to Kuhlenbeck (1922), the vascular supply seems to be about the same as in *Necturus* and markedly poorer than in *Amblystoma*. Particularly, the nearly total absence of capillaries from the cellular portions of the dorsal and lateral pallial areas is notable, a condition the possible significance of which was discussed in the paper on *Necturus* (Craigie, 1940*a*, pp. 403–4). In general, the relative vascularity of the different parts seems to be comparable with that in Caudata.

CONCLUSION

Since the observations show that the cerebral capillary mechanism of *Siphonops* is in all observed particulars similar to that in *Dermophis*, they lend increased probability to the supposition that all members of the order Gymnophiona have an arrangement of the same type. The various considerations brought forward in the discussion of *Dermophis*, and the phylogenetic speculations there indulged in, are given a slightly broader basis, but nothing can at present be added to them.

From the quantitative as well as from the qualitative point of view, the Gymnophiona appear to have a cerebral vascular mechanism comparable with that in the larger Caudata but somewhat less rich than in the more advanced salamanders. It is thus considerably poorer than, as well as architecturally different from, the corresponding apparatus in Salientia.

SUMMARY

1. The capillary bed of the central nervous system of *Siphonops annulatus*, like that of *Dermophis mexicanus*, is composed entirely of slender, independent capillary loops similar to those occurring in Caudata. The component capillaries are unbranched and form secondary side loops only occasionally.

2. As in Caudata, such capillary loops are confined strictly to the central nervous organs.

3. The conditions in *Siphonops* and *Dermophis*, being similar, may probably be regarded as representative for the Gymnophiona in general and hence are suggestive of speculations regarding the ancestral tetrapods.

4. The capillaries of *Siphonops* are considerably wider than those of *Dermophis*, while the size of the erythrocytes in the former exceeds that in the latter even more markedly and is comparable with that in *Cryptobranchus alleganiensis*.

5. The richness of the vascular supply in the hindbrain is intermediate between that in *Necturus* and that in *Amblystoma tigrinum*. The forebrain, however, despite greater specialization, is no more richly supplied than in *Necturus*. The extreme poverty of cellular regions as compared with fibrous parts in the forebrain, which is marked in Caudata, is conspicuous also in *Siphonops*.

REFERENCES

Craigie, E. Horne (1938*a*). *Proc. Amer. Phil. Soc.* **78**, 615.
—— (1938*b*). *Science*, **88**, 359.
—— (1938*c*). *Res. Publ. Ass. Nerv. Ment. Dis.* **18**, 3.
—— (1938*d*). *Trans. Roy. Soc. Can. Sec.* v, Ser. 3, **32**, 43.
—— (1938*e*). *J. comp. Neurol.* **69**, 453.
—— (1939). *Proc. Amer. Phil. Soc.* **81**, 21.
—— (1940*a*). *Proc. Amer. Phil. Soc.* **82**, 395.
—— (1940*b*). *J. Morph.* **67**, 477.
Gulliver, G. (1875). *Proc. Zool. Soc. Lond.* p. 474.
Herrick, C. J. (1935). *J. Comp. Neurol.* **61**, 297.
Kuhlenbeck, H. (1922). *Jena. Z. Naturw.* **58**, 453.
Roofe, P. G. (1935). *J. Comp. Neurol.* **61**, 257.
Scharrer, E. (1939). *Anat. Rec.* **75**, 319.
Schöbl, J. (1882). *Arch. mikr. Anat.* **20**, 87.
Smith, H. M. (1925). *Biol. Bull. Wood's Hole*, **48**, 347.
Sterzi, G. (1904). *Anat. Hefte*, I Abt., **24**, Heft 74.

THE EARLY STAGES IN THE DEGENERATION OF CUTANEOUS NERVE FIBRES

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In a recent paper (Weddell, 1941) a descriptive account was given of the pattern of cutaneous innervation in the rabbit's ear, based on a study of preparations in which the nerve fibres and their endings had been stained intravitally with methylene blue. The same method has been employed for the study of the finer changes which occur in the cutaneous nerve fibres during the early stages of nerve degeneration. The investigation anew of a subject which in the past has occupied the attention of so many eminent neuro-histologists is believed to be justified by the mode of application of the methylene-blue technique. As already reported, it has been found possible by this method to stain uniformly and regularly all the nerve fibres in the rabbit's ear and, by the examination of large total preparations, to follow in continuity the course of individual fibres from their origin in nerve trunks at the base of the ear to their termination in the skin and in the subcutaneous tissues. Thus the process of degeneration can be studied simultaneously throughout a considerable extent of each nerve fibre. Further, by focusing attention on the small subcutaneous fasciculi containing perhaps not more than two or three nerve fibres, it is possible to eliminate complicating and disturbing factors which obscure the reaction of the axone and its neurilemma sheath, such as the fibroblastic activity which may occur in the epineurium and perineurium of larger nerve trunks, the concomitant reaction of the vascular tissues related to blood vessels and lymphatics in such trunks, and the reaction of surrounding tissues in which the trunk may be situated.

Lastly, it may be presumed that vital staining with weak solutions of methylene blue and the direct examination of whole preparations are likely to provide a more accurate picture of the actual changes which occur in the intact living tissue than could possibly be obtained by the more commonly employed silver methods. For it may be argued that the latter perhaps involve not a little distortion, and also the production of artefacts, by reason of the treatment of the tissues with the fixatives and various reagents employed and in some instances with the processes required for embedding and sectioning.

In all cases, however, the methylene-blue picture has been checked and controlled in this study by the application of silver impregnation methods of sections of the same material, and this has permitted a comparative study of the two methods which are of complementary value. The silver techniques chosen, modifications of the Bielschowsky-Gros and Schultze-Stöhr methods, are those which have been used for some years for critical histological work.

These methods involve little tissue shrinkage, and artefacts are believed to have been reduced to a minimum. Frozen sections have been used in order to avoid artefacts due to embedding and the cutting of thin sections.

Since neither methylene-blue staining nor silver impregnation directly demonstrates the structure of, and changes which take place in, the myelin sheath, it was considered advisable to stain specifically for this substance in selected cases. A modified Spielmeyer-Hortega stain was used on frozen sections from normal and operated animals.

The results of this investigation have been recorded photographically so that, as far as possible, detailed facts are presented objectively apart from the interpretations which may be placed upon them.

MATERIAL AND METHODS

Material. Skin from the dorsum of the ear of rabbits was used. It was taken from ears which appeared macroscopically normal and from ears at intervals respectively of 12, 24, 48, 96, 168 and 336 hr. after section of the dorsal ear nerve at the base of both ears in twenty-eight rabbits. Owing to the close relationship in pattern between the venous system and nerve trunks in the ear, skin was not removed from those ears which had been used for making intravenous injections. One ear was used for methylene-blue staining and the opposite ear for silver impregnation.

Histological methods. Methylene blue. A 0·015 % solution of B.D.H. standard stain in 0·9 % sodium chloride A.R. containing 1 % procaine hydrochloride was used, the time allowed for staining after injection being three-quarters of an hour. Further details of the method have been given previously (Weddell *et al.* 1940; Weddell, 1941). After staining, the skin of the dorsum was gently stripped from the cartilage and placed in an 8 % solution of ammonium molybdate in which it was allowed to remain for 12 hr. at a temperature of 0° C.; on four occasions the skin was not stripped from the cartilage but was allowed to remain undisturbed until the fixation in alcohol had been completed. The skin was then washed, fixed in absolute alcohol, cleared in benzol (cryst.) and stored in methyl salicylate. For work at high magnifications selected pieces of skin were occasionally fixed in formalin after washing, and frozen sections cut; these were counterstained, dehydrated, cleared, and mounted in balsam in the usual way.

Silver. The distal half of the ear was placed in a solution of 10 % formalin containing 2 drops/100 c.c. of pyridine, in which it was allowed to remain for 3–5 days. Large frozen sections were cut and washed for approximately 2 hr. in distilled water. They were then placed in a 20 % solution of silver nitrate for between 5 and 30 min. according to the rapidity with which the slight yellow colour change took place at this stage. The sections were transferred to a series of Petri dishes containing a 3 % solution of formalin in tap water; progressive baths were used with agitation until in the final bath no precipitation occurred. After this it was sometimes found advisable to wash the sections in distilled

water to which had been added 2 drops/100 c.c. of ammonia 0·880. The sections were then placed in an ammoniacal silver reducing bath made by adding ammonia 0·880 to a 20 % solution of silver nitrate until the precipitate which comes down was just dissolved; this reduction was always controlled under the microscope. After reduction the sections were placed in an Eastman Kodak E.K.F–5 acid hypo fixing bath in which they remained from ½ to 1 min., and they were then washed, dehydrated in 96 % alcohol, cleared in creosote, and mounted in balsam. On two occasions the ear to be used for silver impregnation was first fixed by an intravenous injection of formalin made through the heart.

Myelin stain. The distal half of the ear was fixed in a solution of 10 % formalin for 3 days and then washed in running tap water for 6 hr. Frozen sections were cut and transferred to a 5 % solution of iron alum for 6 hr. at a temperature of 60° C.; the sections were then washed for half an hour in tap water, after which they were transferred to a solution of iron haematoxylin in which they remained for 12 hr. The iron haematoxylin was made by adding 5 c.c. of a 10 % alcoholic solution to 100 c.c. of tap water. The sections were then washed briefly and differentiated in a 5 % solution of iron alum. After immersion for a few seconds in a solution of 1 in 500 potassium permanganate they were placed in a 5 % solution of oxalic acid in tap water until they assumed a yellow brown coloration; they were then transferred to a solution of 20 drops of ammonia 0·880 in 100 c.c. tap water until they turned blue. The sections were finally washed for 6 hr. in tap water, dehydrated, cleared and mounted in the usual way.

HISTOLOGICAL OBSERVATIONS

Normal skin. In skin from the dorsum of a normal ear the large nerve trunks contain both medullated and non-medullated fibres of varying diameters (Pl. 1, fig. 8). With the methylene blue and silver methods employed in the present study the medullated nerve fibres can only be distinguished from the non-medullated fibres by their larger diameters and by the presence of nodes of Ranvier, where the soldering discs of Ranvier and the double bracelets of Nageotte can be clearly seen (Pl. 1, figs. 1, 9). On occasions the faint outline of the myelin sheath can be observed and this is probably not indicative of pathological change unless concomitant axonic deviations from the normal can be observed. The reinforced *Innenscheide* of Mönckeberg and Bethe described by Nageotte (quoted by Cajal, 1928) can also be detected in certain fibres. Occasionally fibres of small diameter are seen springing from nodal points and accompanying the parent trunk as far as the cutaneous nerve plexus where both fibres pursue remarkably similar courses (Pl. 1, fig. 10). This appearance is very suggestive of *collateral regeneration*, which Nageotte (1932) describes as being of interest because traces of it can always be found in normal animals.

The internodal portion of the axone of normal medullated fibres is smooth and homogeneous; on occasion, however, its diameter fluctuates considerably

along its course between successive nodes. This change in diameter occurs among the fibres of bundles in which other axones are unaffected and in which no tissue displacement can be observed; moreover, it is seen in both methylene blue stained specimens and in those impregnated with silver. In a number of cases the change in diameter is due to indentation of both medullary sheath and axis cylinder by the nuclei of the Schwann sheath (Pl. 1, figs. 2, 15). There is also a progressive diminution in the average diameter of the nerve fibres which can be seen if they are traced in continuity from the nerve trunks to their terminations. This change in diameter through the trunk is a gradual one and can only be detected by successive measurements. When the fibres enter the cutaneous nerve plexus the change is more rapid (Pl. 1, fig. 4). At a point of dichotomy within the plexus, however, the fibre does not divide into two fibres having half the diameter of the parent trunk; on the contrary, it is often difficult to tell by direct inspection that there has been any change in calibre (Pl. 1, fig. 11).

The non-medullated fibres have smooth homogeneous axis cylinders; local changes in diameter are not observed, but a general decrease in diameter takes place as the fibres are traced to their destination similar to that seen in the medullated fibres (Pl. 1, fig. 3).

The axis cylinders of all fibres remain smooth and unbeaded until the fibres are reached which give rise to the subepidermal nerve nets and free endings, or to the nerve nets and free endings related to blood vessels. From this point the fibres are stained only in the methylene-blue preparations and have a moniliform appearance (Pl. 1, figs. 12, 13). In normal fibres the separate beadings are in all cases linked by fine stained threads. It has been suggested that such beading is an artefact and due to the effect of the stain; it is significant, however, that workers using other silver impregnation methods report similar appearances; moreover, it has been demonstrated that such stained fibres can conduct impulses (Woollard *et al.* 1940) and they have been figured as occurring in living unstained nerve fibres in the tail of the tadpole (Speidel, 1933).

The Schwann nuclei are usually visible after silver impregnation methods which specifically demonstrate the axis cylinder; the Schmidt-Lantermann clefts, annular rings, infundibular membranes, and Schwann cytoplasmic veils (Cajal, 1928), on the other hand, can only be seen after a modification of technique which involves the elimination of pyridine from the fixing fluid, and an increase in the percentage of formalin in the baths which follow the first silver solution (Pl. 1, figs. 5–7).

Specific staining of the myelin sheath shows, in addition, that the Schmidt-Lantermann clefts are present only along the course of the larger myelinated fibres, where nodes of Ranvier are clearly evident, and the faintly outlined Schwann nuclei can be seen indenting the sheath at intervals (Pl. 1, figs. 14, 15). Only among the larger myelinated fibres of the nerve trunks, or along fibres passing between such trunks, do the myelin sheaths preserve this classical

form. Among the fibres of the cutaneous nerve plexus the myelin sheath is smooth and homogeneous, but neither nodes of Ranvier nor Schmidt-Lantermann clefts can be seen; the myelin sheath, however, is often in the form of short isolated segments, the intersegmental gaps bearing no resemblance to nodes of Ranvier (Pl. 1, fig. 16). Occasionally fine myelinated fibres leaving the plexus on their way to hair follicles lose their myelin sheath and travel naked for some distance, after which they may again become surrounded by short isolated segments of myelin before reaching their destination (Pl. 1, fig. 17). Such findings have been previously reported by Speidel (1935), working on living nerve fibres in the tail of the tadpole, and by Weddell & Harpmann (1940), in methylene-blue preparations of tendon from a rabbit. The majority of the myelinated fibres which supply hair follicles do not lose their sheath until they commence breaking up into their terminal arborizations (Pl. 1, fig. 18). As the fibres decrease in diameter through the cutaneous nerve plexus towards their terminations they lose their myelin sheath. Many fibres lose their sheath on entering the cutaneous nerve plexus itself. This is in accordance with Duncan's (1934) theory of progressive myelination with increasing diameter of nerve fibres.

Occasionally in unoperated preparations normal fibres are seen in which the axis cylinder is abruptly interrupted for a short interval. Such effects are probably artefacts due to sudden stretching of the skin at the time of injection of the methylene blue or to excessive stretching of the skin during its removal from the cartilage, for they are not seen in silver preparations or in methylene-blue preparations in which stripping is delayed until after fixation or in which the dye is injected slowly with a fine needle.

12 *hr. after nerve section.* Signs of nerve degeneration are apparent throughout the dorsum of the ear. In the nerve trunks and among fibres of largest diameter the changes are least marked, and a small proportion (less than one-third) of fibres of all diameters are indistinguishable from the normal. These fibres which are apparently 'resistant' to early degenerative changes can be traced to their terminations around hairs, around blood vessels, and in subepithelial nerve nets. Fibres which in main nerve trunks display only the slight signs of degeneration show more pronounced changes distally when they are traced to the cutaneous nerve plexus.

The earliest signs of degeneration 12 hr. after nerve section in the large medullated fibres of the nerve trunks are irregularities in the staining of the axis cylinders, which usually take the form of a minimal degree of fibrillation at irregular intervals along the course of the fibre. This is seen both in methylene-blue and silver preparations (Pl. 2, figs. 19, 21). In addition, in methylene-blue preparations the medullary sheath becomes faintly outlined in blue and either appears structureless or, more rarely, contains a network of blue beaded threads (Pl. 2, fig. 22). Annular rings are visible in such fibres and irregular blue formations suggesting morbid Schmidt-Lantermann clefts (Pl. 2, fig. 23). These changes are most marked immediately adjacent to the nodes of Ranvier,

which themselves, however, show no change. Silver impregnation shows that there are irregularities affecting the outline of the Schmidt-Lantermann clefts and the annular ring and infundibular systems; the clefts are more ring-like and their contour irregular in outline (Pl. 2, fig. 24).

Among the thinner medullated and non-medullated fibres in the nerve trunks changes are only apparent in the axis cylinders. These either appear as a series of granular, darkly-stained or impregnated fragments, or else as a series of irregular and occasionally fibrillated fusiform swellings along the course of the fibres (Pl. 2, fig. 20). These changes are probably a matter of degree rather than representing a qualitative difference, for both types of change can be seen in the same fibre at different positions along its course; the more severe granular changes occur towards the cutaneous nerve plexus.

In the fibres of the cutaneous nerve plexus the degenerative changes are more easily recognized because for the most part they are more severe. They are similar to those seen among the finer fibres of the nerve trunks. A greater proportion of the axis cylinders have become fragmented and only a few can be seen in which the fusiform swellings remain interconnected (Pl. 2, figs. 25, 28). The same axonic changes occur in both medullated and non-medullated fibres. In the case of a number of non-medullated fibres, usually those leaving the plexus on their way to blood vessels, the changes are somewhat different; in addition to a fragmentation of the axis cylinder, which may be more pronounced than in other neighbouring fibres, the nuclei and cytoplasm of the Schwann syncytium become outlined, the nuclei being more darkly stained than the cytoplasm. This effect can be seen both after methylene blue staining and silver impregnation (Pl. 2, figs. 26, 27). The thicker nerve fibres in the nerve trunks from which such fibres arise show variable degrees of degenerative change which increase in severity as they are traced into continuity with the fibres in question. They are not precociously degenerated as compared with other neighbouring non-medullated fibres in the nerve trunks. The fine beaded fibres of the subepithelial nerve nets and those innervating blood vessels show no morphological change at this stage, nor do the beaded fibres arising from the degenerated segments of non-medullated fibres just described.

The terminals of the fibres around the hairs show signs of degeneration which correspond with those of the fibres from which they arise.

The changes which have just been described in the peripheral stump 12 hr. after section of a cutaneous nerve correspond exactly with the descriptions of early stages in nerve degeneration given by Cajal (1928), Speidel (1933, 1935) and a number of other workers. Our observations confirm the statement that at this stage the finer nerve fibres in general suffer more severe degenerative changes than the thicker fibres, and they have shown that in the ear of the rabbit there is a progressive diminution in the size of the fibres as they are traced from nerve trunks to their terminations. In addition it is confirmed from a study of these degenerative changes that there is in any one area of the skin a short range spectrum of fibre sizes related to each type of nerve termination.

Cajal (1928) has suggested that methylene blue stains the so-called neuroplasm or more liquid phase of the axis cylinder, while silver impregnates the neurofibrillae or more solid phase. The early degenerative changes demonstrable with methylene blue certainly suggest that there is a more liquid phase in the axis cylinder, for, some time after section of the nerve, this appears to diffuse into the sheath, rendering its outline, and at times its structure, visible by blue staining, while at the same time the axis cylinder becomes proportionately less brilliantly stained; this conjecture is further supported by the fact that, after section of a living methylene-blue-stained nerve fibre under direct vision in the ear of the rabbit, a blue substance can be seen gradually to diffuse from the ends of the cut fibre into the surrounding tissues, and the axis cylinders become considerably paler in the region of the cut; they remain visible, however, until the colour fades from the neighbouring unoperated nerve fibres. In this connexion it may be noted that the best staining of axis cylinders with methylene blue actually occurs immediately following nerve section (i.e. before diffusion into the surrounding tissues begins to occur); this suggests that the very earliest degenerative changes are of a physical nature leading to a marked increase of the permeability of the dye in relation to the axis cylinders. This inference is in accord with the observations of Speidel (1935) who states that after cutting a living nerve fibre in the tail of a normal tadpole immediate and rather violent effects are seen. For instance, during the first hour currents of fluid are observed in the axis cylinder region, and vacuole formation between the axis cylinder and myelin can be seen taking place.

It is clear that early degenerative changes involve a swelling of the axis cylinder which may take on a fibrillary appearance, and it may be that, as suggested by Marinesco (1907), one of the earliest signs of degeneration in a fixed preparation is shown by visible fibrillation of the axis cylinder. Since the fibrillation can be directly demonstrated by both methylene blue staining and silver impregnation, it can hardly be the case that, as suggested by Cajal, the former method gives as it were positive and the latter negative images which are mutually complementary, the one staining the liquid phase and the other impregnating the more solid phase in the axis cylinder. The demonstration by both silver and methylene-blue methods of the Schwann syncytium in occasional degenerating non-medullated fibres is also difficult to explain on such a basis. The latter finding, however, certainly suggests that the particular silver method employed can be more closely compared with a stain than with a surface precipitation. It is significant that the finer the grain in a silver preparation the more closely the results compare with methylene blue staining. The fact that neurofibrillae in normal nerve fibres are not demonstrated by these methods suggests that, if they are indeed present as morphological entities, they are very closely packed in the normal axis cylinder. Speidel (1935) states that neurofibrillae are optically invisible in normal living unstained nerve fibres in the tail of the tadpole but that structures resembling fibrillae become apparent during the early stages of degeneration.

24 *hr. after nerve section.* In the nerve trunks there are still occasional fibres of all sizes which show no sign of degeneration, but the number of such fibres has declined by approximately half as compared with those seen 12 hr. after nerve section. The majority of fibres show progressive signs of degeneration which are in an inverse ratio to their axis cylinder diameters (Pl. 2, figs. 29–33). The large medullated fibres show a general swelling of the axis cylinder which may be accompanied by the appearance of a progressive fibrillation. The myelin sheaths are more clearly outlined by staining with methylene blue and their contour slightly irregular. The outlining is apparently due to a diffusion of a more fluid phase of the axis cylinder into the myelin sheath (Pl. 2, fig. 29). The axis cylinders of the thinner medullated fibres usually undergo granular fragmentation (Pl. 2, fig. 30); in addition, segmentation of the faintly outlined myelin sheath can be seen among the smallest fibres, each segment containing either a piece of swollen fibrillated axis cylinder or granular axis cylinder remnants (Pl. 2, fig. 33). These myelin segments correspond precisely with the *ellipsoids* or *digestive chambers* of Cajal-(1928). A small number of the large medullated fibres undergo 'precocious' degenerative changes; in such cases there is no fibrillation or general swelling, but a vacuolation and granular disorganization of the axis cylinder (Pl. 2, fig. 32). Schmidt-Lantermann cleft outlines can be clearly seen in connexion with the *ellipsoids* of some of these 'precociously' degenerated fibres (Pl. 2, fig. 31).

In the cutaneous nerve plexus the changes are similar but in general more severe. In the majority of cases, the medullated fibres are in the form of *digestive chambers* with considerable gaps between the axis cylinder remnants which have either contracted into short swollen segments or have commenced to disintegrate (Pl. 2, fig. 35). The axis cylinders of other fibres have undergone fragmentation but have not reached the stage of *ellipsoid formation* (Pl. 2, fig. 34). In the case of methylene-blue-stained preparations the non-medullated fibres of the plexus are in many instances represented by stained granular nucleated threads containing only an occasional segment of recognizable axis cylinder; the ends of such fibres which lead either to cutaneous nerve nets or to beaded fibres around blood vessels terminate in granular blobs and can no longer be traced to their final destinations (Pl. 2, fig. 36). The moniliform fibres of the subepithelial nerve nets, and those innervating blood vessels, show some increase in the size of the individual beads in a few places, but the majority of the fibres appear quite normal. The nets can, however, no longer be traced into continuity with fibres of the cutaneous nerve plexus. The terminals ending around the hairs show changes similar to those of the fibres from which they arise.

48 *hr. after nerve section.* All nerve fibres now show signs of degeneration in some part of their course, but a small number of fibres of all sizes still lag behind the majority in the degree of change which they display.

In the nerve trunks many of the axis cylinders of the large myelinated fibres have increased in diameter with progressive fibrillation so that they now

almost fill their myelin sheaths (Pl. 3, fig. 40). Sometimes the axis cylinder is swollen up within a faintly outlined myelin sheath without the appearance of fibrillae; the margins of such an axis cylinder are indistinct (Pl. 3, fig. 38). There is yet another variation in which the axis cylinder is swollen but at the same time has become finely granular; in such a case the myelin sheath appears reticulated and the annular rings and remnants of Schmidt-Lantermann clefts are outlined by the stain (Pl. 3, figs. 37, 38). In the large myelinated fibres at this stage of degeneration, details of the double bracelets of Nageotte (which are now clearly spinous, Pl. 3, fig. 41) and the reinforced *Innenscheide* of Mönckeberg and Bethe are particularly evident after staining with methylene blue (Pl. 3, fig. 39). These structures, together with the soldering disc of Ranvier, appear to bind the fibrillae at the nodes and this seems to delay the disintegration of the axis cylinder at these points. The discs of Ranvier are no longer demonstrated by silver methods at this stage. The finer myelinated fibres of the trunks show further signs of axonic disintegration, and *digestive chambers* are beginning to form along the course of a number of these fibres (Pl. 3, figs. 42, 43).

In the cutaneous nerve plexus the majority of the myelinated nerve fibres show *digestive chamber* formation right up to the terminals around the hairs (Pl. 3, figs. 45, 46), while the non-myelinated fibres show either granular fragmentation of the axis cylinders or granular fragmentation with staining of the Schwann syncytium (Pl. 3, fig. 44).

Among the beaded fibres of the nerve nets changes are now evident. The size of the individual beads has increased in many cases, and connecting links between them are no longer visible. A number of the fibres, however, still appear normal (Pl. 3, fig. 47).

96 *hr. after nerve section.* In the nerve trunks all nerve fibres show some signs of degeneration. A few of the largest medullated fibres appear only mildly affected towards the base of the ear, but when traced distally these show increasing signs of change (Pl. 3, figs. 48–55). The majority of the medullated fibres are in the form of large *digestive chambers* which contain either granular fragments of axis cylinder or small twisted segments (Pl. 3, fig. 50 and Pl. 4, figs. 58, 63). In a smaller number of fibres, while there are severe disintegrative changes of the axone, the faintly outlined myelin sheath still remains unsegmented. The only fibres which remain resistant to degenerative changes are a small number of non-medullated fibres which can be followed to the remnants of nerve nets around blood vessels; these fibres appear normal in the trunks in the greater part of their course but show unmistakable signs of degeneration after leaving the trunks (Pl. 4, fig. 57). During their course in the trunks, however, all these fibres show at intervals isolated fusiform swellings apparently enclosed by a splitting of the axis cylinder; such swellings are not seen in normal fibres (Pl. 3, fig. 56).

In the cutaneous nerve plexus about half the number of fibres have disappeared (Pl. 4, figs. 59, 60). The remaining fibres are represented by irregularly

shaped, faintly outlined, digestive chambers containing a decreasing number of axis cylinder remnants, and by a few non-medullated fibre remains consisting of granular axones within faintly outlined sheaths (Pl. 4, figs. 61, 62). Most of the axis cylinders of the nerve nets have disintegrated and disappeared, but here and there a short and apparently normal segment can be seen which ends in a large irregularly outlined granular drop. Occasionally an isolated segment of nerve can be seen ending around a blood vessel similar to the segments of the cutaneous nerve net which are still present. Specific staining for myelin at this stage confirms all the statements which have been made concerning the changes in the myelin sheath as seen after methylene-blue staining (Pl. 4, figs. 64–68).

At this stage of nerve degeneration it has been noted that, after staining with methylene blue, the nuclei of the capillary endothelium and a number of connective tissue cells become clearly outlined in blue. It is suggested that this phenomenon is possibly due to the general diffusion through the tissues of the stainable substance of the disintegrated axis cylinder. It is clear that such a diffusion takes place during degeneration, but whether the products of diffusion are retained in the surrounding tissues is a matter of conjecture. It may be that the more generalized staining effect is due to the decreased vitality of the tissues of the ear, and is an expression of the so-called 'trophic' change which follows denervation. In any case, there is undoubtedly a metabolic change in the surrounding tissues which follows nerve section and which can be demonstrated by vital staining with methylene blue.

These accounts of slightly later stages in the degeneration of cutaneous nerve fibres again closely follow those of Cajal (1928). In addition, we have shown not only that vaso-motor fibres are comparatively resistant to degenerative changes, but that fibres of the cutaneous nerve nets are also resistant. We have also shown that the nodes of Ranvier are usually clearly recognizable in methylene-blue preparations 48 hr. after nerve section when they can no longer be impregnated by silver methods. Cajal (1928) recognized this difficulty with silver methods and says with reference to the nodes of Ranvier at this stage that 'they do not attract the silver nitrate at all'.

It has been further demonstrated that, although degenerative changes probably occur simultaneously along the entire length of a peripheral stump, the type and degree of change are related to a great extent to the diameter of the axis cylinder. Thus at some points the fibre may appear quite normal and at others show severe degenerative changes. Cajal believed that changes were the same throughout the whole length of a degenerated fibre. While this statement is almost certainly true for the sciatic trunk, we have shown that it is not the case among cutaneous nerve fibres.

168 *hr. after nerve section.* In the main nerve trunks about one-third of the remnants of the smaller nerve fibres have disappeared (Pl. 4, fig. 69); the remainder are in the form of *digestive chambers* which contain a variable amount of axonic debris, or granular axonic segments which are in various stages of

disintegration (Pl. 4, fig. 70). Similar *digestive chambers* and axonic remains are shown by silver impregnation; in addition, this method demonstrates an increasing number of Schwann nuclei, lying within what appear to be syncytial bands or cylinders which have replaced the original nerve fibres. These nuclei are densely packed with darkly impregnated granules (Pl. 4, fig. 73). The Schwann nuclei belonging to fibres which still have axonic remains present are less elongated and considerably paler; the cytoplasm is abundant and faintly impregnated with silver. In the small nerve trunks some axonic remnants are still visible and can be traced as far as the cutaneous nerve plexus where they cease (Pl. 4, figs. 71, 72). The remnants of the fibres of the cutaneous nerve plexus and nerve nets have disappeared. Silver impregnation shows that only Schwann nuclei within syncytial bands or cylinders remain, and that all traces of myelin and axonic substance have disappeared. After staining with methylene blue a further increase in general nuclear staining is seen throughout the tissues of the ear; the nuclei of the capillary endothelium and fibroblasts are particularly evident.

336 *hr. after nerve section.* The number of nerve fibre remnants in the main nerve trunks is now about half that seen 168 hr. after nerve section (Pl. 5, fig. 74). The remains are all within digestive chambers and these contain a variable amount of axonic substance. Some chambers are clearly outlined in blue and contain numerous darkly stained particles, some of large size (Pl. 5, fig. 75), others only faintly outlined and containing no granules whatever. If degenerating medullated fibres are followed in continuity for some distance it becomes clear that the majority of the particles which are stained in the digestive chambers, the spheres of Elzholz, are fragments of a more solid phase of the degenerating axis cylinder.

Silver impregnation presents a similar picture with regard to the digestive chambers and axonic remnants and, in addition, it is possible to impregnate the nuclei and cytoplasm of the Schwann syncytium. In many cases the syncytium shows a decrease in the number of nuclei, which are smaller, more elongated, and less granular compared with those present 168 hr. after nerve section. The cytoplasm seems to contain fine longitudinal striations, which, however, according to Cajal and Nageotte, are caused by overlying connective tissue fibrillae of the closely investing sheath of Key and Retzius and are not fibrillations within the Schwann cytoplasm itself (Pl. 5, fig. 76).

The methylene-blue method shows that generalized tissue staining has again increased in amount but the nuclei and cytoplasm of the Schwann syncytium remain unstained. A number of the sebaceous gland cells which normally take up the stain, however, now fail to do so.

From this study of the morphology of the normal nerve fibre and the changes which it undergoes during the early stages of degeneration, it is clear that the methylene-blue staining method employed is capable of giving critical histological pictures which are comparable with those obtained by carefully selected silver methods. The two methods are indeed complementary, with the

exception that the finer terminal ramifications of the nerve fibres can only be demonstrated with constancy by methylene-blue staining. Methylene blue, when staining intravitally in normal skin and during the early stages of nerve degeneration, is virtually specific for the biochemical constituents of the axis cylinder, and while a proportion of these substances are still present in the nerve fibres they continue to stain with methylene blue. The greater diffusibility of a part of the axis cylinder complement enables a number of the changes in the myelin sheath to be seen during the course of degeneration.

In this connexion it is of interest and importance to emphasize that the observations made by Speidel (1933, 1935) on the behaviour after section of living nerve fibres in the tail of the tadpole compare very closely with the present observations on methylene-blue stained material from the mammalian ear. Particularly do we agree with the statement of Speidel that in a normal nerve fibre no neurofibrillae can be seen, but that fibrillation often becomes apparent during early stages of degeneration. Our preparations likewise show that the fibrillae are never so numerous or so clearly defined as those seen after the use of special neurofibrillar impregnation methods. We have also demonstrated that a number of non-myelinated fibres may be surrounded by a single syncytial Schwann sheath, thus confirming the findings of both Nageotte (1932) and Speidel (1935).

As already stated, it is in general impossible to stain the course of degenerating nerve fibres with methylene blue after all the axis cylinder remnants have been resorbed. Thus over the greater part of the dorsum of the ear 336 hr. after section of the dorsal ear nerve only a few axis cylinder remnants in main nerve trunks take up the stain, and the cutaneous nerve plexus has apparently disappeared. In certain regions, however, where the territories of adjacent cutaneous nerves overlap it is possible to outline the remnants of degenerated fibres of the cutaneous nerve plexus with the dye. Such *intermediate zones* can be found in the ear of the rabbit. After section of the main dorsal ear nerve the greater part of the dorsum becomes completely denervated, but a small portion of the medial margin usually escapes as it is innervated by a nerve trunk which enters the base of the ear close to the margin. The *autonomous zone* of this nerve is extremely small while the *intermediate zone* is somewhat larger. Within this *intermediate zone*, 336 hr. after section of the dorsal ear nerve, sheaths of degenerated nerve fibres of the cutaneous nerve plexus, containing irregular oval unstained masses and usually devoid of definitive axis cylinders, become brilliantly outlined with blue (Pl. 5, fig. 79). In other sheaths small dark particles can be seen in the position of the axis cylinder, and in still other sheaths there are segments resembling fine threads of axis cylinder and a minimal amount of debris (Pl. 5, fig. 78). There are stained nuclei in the walls of the sheaths. This reaction is confined to sheaths related to the cutaneous nerve plexus. These sheaths can be traced into continuity with nerve trunks in which there are both normal and a few degenerating fibres similar to those seen in the central trunks (Pl. 5, fig. 77); the staining of the sheaths ceases abruptly when

the nerve trunks are reached. This staining reaction does not occur in the *autonomous zone* where the nerve fibres appear normal, or in the denervated area where the cutaneous nerve plexus has apparently disappeared. No clear-cut growth cones are seen entering the sheaths from the trunks indicative of regeneration from the point of section, but sometimes apparently normal axis cylinders in the cutaneous nerve plexus enter the sheaths and penetrate into them for short distances (Pl. 5, fig. 80). Growth cones from fibres of the cutaneous nerve plexus are occasionally seen in the tissues surrounding the stained sheaths (Pl. 5, fig. 81). The sheaths observed are in every way similar to the bands of Büngner described by Cajal (1928) and those depicted by Nageotte (1932). Whether such appearances are indicative of early stages in a new growth of fibres derived from the remaining normal axones within the cutaneous nerve plexus, a precocious regeneration from the point of nerve section, or merely a different chemical change during the course of degeneration in such zones, is not clear. However, suggestions indicative either of local new growth from normal fibres within the plexus or precocious regeneration from the point of section are very strong.

Observations by Speidel (1933, 1935) are of great interest in connexion with these findings in *intermediate zones*. Working on living nerves in the tail of the tadpole he observed that, after section of a small non-myelinated fibre, granular degeneration rapidly occurred along the distal stump which soon disappeared. When no regeneration took place along this path, collateral sprouts from near-by normal nerves grew out into the denervated zone, usually following entirely new routes. He also observed in one case that section of a single myelinated nerve fibre was followed by the genesis and growth in a proximal direction of a branch from a near-by non-myelinated fibre which eventually joined the degenerating fibre, thus offering a choice of pathways for the newly regenerating fibres advancing from the cut region. In this particular case the collateral path was chosen.

SUMMARY OF HISTOLOGICAL OBSERVATIONS

The following summary may be given of the findings in connexion with the Wallerian degenerative changes in the cutaneous nerves from the dorsum of the rabbit's ear between 12 and 336 hr. after nerve section, as seen in methylene-blue stained specimens:

Axis cylinder. Large medullated fibres. The earliest changes in large medullated fibres are either minimal degrees of fibrillation, which appear in fusiform swellings at irregular intervals along the course of the fibre, or a general swelling of the axis cylinder without fibrillation. At the same time a more fluid portion of the axis cylinder appears to diffuse into the myelin sheath which becomes outlined in blue. One of two things now happens; either the fibrillae or swollen axis cylinders commence to fragment, the particles scattering throughout the myelin sheath, or the fibrillae or swollen axis cylinders break up into segments which become twisted but remain more or less in the

centre of the myelin sheath. The axonic remains then slowly disintegrate, the last particles to remain being those fibrillae or axis cylinders which segment but do not fragment. At the nodes of Ranvier the same changes occur but here they are always delayed. The delay is apparently due to the soldering disc of Ranvier, the bracelets of Nageotte, and the reinforced *Innenscheide* of Mönckeberg and Bethe. These structures appear to bind the axis cylinder mechanically and to delay its disintegration at the nodes. Silver impregnation confirms these findings.

Axis cylinder. Small medullated fibres. These are affected before the large fibres and degeneration proceeds more rapidly. The changes are quite similar, with the exception that fibrillation is an uncommon feature. The fusiform swellings occur as described above, but fibrillation cannot be distinguished within them. Silver impregnation again confirms these findings.

Axis cylinder. Non-medullated fibres. In general these are affected before all other fibres. The axis cylinder undergoes granular fragmentation, and during the course of its disintegration a more liquid portion of the axone may cause the Schwann syncytium and nuclei to become stained for a brief period. The solid phase of the axis cylinder then undergoes rapid resorption, after which the fibre is no longer visible in methylene-blue preparations. These findings are confirmed by silver impregnation.

Myelin sheath. This is never directly stained by methylene blue, but many of the changes which it undergoes can be followed by the apparent diffusion into it of a more fluid phase of the axis cylinder. In the early stages the myelin seems to undergo a certain amount of dislocation so that a reticular network appears throughout the sheath, and the Schmidt-Lantermann clefts and annular rings can be outlined by methylene blue staining of the diffused axioplasm. The myelin then breaks into droplets of various sizes which form the so-called *digestive chambers* or *ellipsoids* in which the remnants of the axis cylinder disintegrate. Silver impregnation confirms the findings with regard to the Schmidt-Lantermann clefts and associated structures, and specific staining for myelin confirms the findings made after methylene-blue staining with regard to the changes which this sheath undergoes during the course of the early stages of degeneration.

Schwann cells. Neither the nuclei nor the cytoplasm of these cells are stained by methylene blue except during the degeneration of certain non-medullated fibres and in the cutaneous nerve plexus in *intermediate zones.* Silver impregnation shows that from 96 until 168 hr. after nerve section the Schwann nuclei proliferate and come to surround the *digestive chambers* in which the axone is disintegrating. These chambers are gradually resorbed. 336 hr. after nerve section all remnants of myelin and axis cylinder remains have disappeared from the fibres of the cutaneous nerve plexus. In such regions the Schwann nuclei have ceased to multiply, the syncytial cytoplasm has diminished in amount, and the original course of the nerve fibres is represented by shrunken sterile strands or cylinders of protoplasm containing small

elongated nuclei in their walls, and intimately covered by the connective tissue sheath of Key and Retzius which probably gives the sheath its fibrillated appearance.

Precociously degenerating fibres. The fibres are of the large myelinated variety. The axis cylinders undergo a rapid vacuolation and fragmentation and the more fluid portion of the axone rapidly diffuses through the myelin sheath; almost complete disintegration of the more solid phase may be effected before the formation of myelin *ellipsoids* (Pl. 3, fig. 38).

Resistant fibres. A small proportion of fibres of all diameters appear to resist degenerative changes for intervals varying from 12 to 48 hr. and perhaps longer. No fibres, however, resist for longer than 4 days. The most resistant fibres are non-medullated fibres which pass to blood vessels and the non-medullated fibres which compose the nerve nets.

Other degenerated fibres. The sheaths of certain degenerated fibres which are no longer evident in methylene-blue preparations of totally denervated areas are stained by methylene blue in *intermediate zones.*

Pattern of degeneration. The pattern of degeneration of the cutaneous nerves in the rabbit's ear suggests that each group of hairs, blood vessels, or nerve nets is supplied by a small spectrum of fibre sizes. This is in conformity with a previous finding that each unit area of skin is innervated by multiple fibres which approach from different directions (Weddell, 1941). Such variation in the diameter of nerve fibres may possibly be concerned with conduction speeds and perhaps determines the fact that although each unit sensory area is innervated by multiple fibres approaching from all directions, yet the impulses aroused by the appropriate stimulation of each area ultimately reach the central nervous system simultaneously.

THE REACTION OF SCHWANN CELLS TO VITAL DYES

In accounts of the process of nerve degeneration, the opinion is sometimes expressed that the Schwann cells assume active phagocytic functions and, in addition to the activities of histiocytes, play an important part in the ingestion of axonic debris. Such a role may be deemed unlikely considering the ectodermal origin of the neurilemma cells. On the other hand, it may be noted that the view has been held by some embryologists that the pia-arachnoid tissue has an embryological origin similar to that of neurilemma cells, and Woollard (1924) has demonstrated that under conditions of irritation and in the presence of particulate foreign matter the arachnoid mesothelial cells can become mobilized in the form of free phagocytes and then react in a characteristic manner to vital dyes. Experiments were therefore initiated in order to study the reaction of neurilemma cells to vital dyes during the course of cutaneous nerve degeneration.

In a series of rabbits, the animals were injected intravenously or intraperitoneally with a 10 % solution of lithium carmine. One unoperated rabbit was given 15 c.c. intravenously daily for 7 days. Two other rabbits were given

similar doses for 7 and 14 days respectively after the dorsal ear nerves had been cut. Further rabbits were given the same amount of dye intraperitoneally for 4, 7 and 14 days after nerve section. In addition a local injection of dye was made into the skin of the ear of one rabbit 12 days after nerve section. At the conclusion of the periods of injection the ear skin was stained with methylene blue and whole preparations made in the usual way; in addition, counterstained sections of the methylene-blue-stained ear skin, spleen and liver, and counterstained smears of bone marrow, were also prepared.

In no experiment were any dye particles seen regularly in the position of the Schwann cells, and in no case were leucocytes or other connective tissue cells containing dye particles seen regularly along the course of the nerve fibres. Macrophages containing dye particles were clearly seen in the liver, spleen and bone marrow; they were also scattered throughout the subcutaneous tissues of the ear, more particularly in areas in which there was evidence of tissue damage. In the position of local injection of the dye there was a small collection of cells containing dye particles, but no particles were seen regularly along the course of the neighbouring nerve trunks. The absence of leucocytes or other phagocytes is in accordance with the findings of Nageotte (1932) who states that such cells are not found among small bundles of degenerating nerve fibres.

The absence of dye particles in the Schwann cells is perhaps not surprising, for nerve degeneration, as seen in small cutaneous nerve bundles, is in no way comparable with an inflammatory process.

THE PRESENCE OF DEGENERATING AND REGENERATING NERVE FIBRES IN NORMAL SKIN

During the course of this and previous investigations in which skin from the rabbit's ear had been stained intravitally with methylene blue, it was noticed that in the skin of normal unoperated animals a small proportion of nerve fibres in the nerve trunks, and a rather larger number in the cutaneous nerve plexus, commonly show signs of degeneration comparable with those seen at various intervals after nerve section. This was at first thought to be due either to a technical fault in the staining method employed or to relatively slow penetration of the fixative. Degenerating fibres were also seen, however, in the normal thin ear skin in which fixation had been effected by perfusion in addition to immersion, and which had been impregnated by silver. Moreover, similar degenerative changes have been observed in the ears of rabbits kept under careful conditions in separate cages, fed on a normal diet, and in which there was no evidence of trauma. Instructions had been given that the animals should not be carried by their ears, although handling by the ears at some previous time cannot be excluded in any of the cases examined. The possibility of minor degrees of vitamin deficiency in the rabbits examined likewise cannot be excluded, although they had all been fed on liberal supplies of fresh greens since their arrival in the department.

In the nerve trunks of the normal ear a small number of fibres (1–5 %) are observed to be undergoing degenerative changes, and over a wide field in the same preparation all stages in the process can be seen, including *digestive chamber* formation (Pl. 5, figs. 82–85). Regenerating fibres (1–5 %) are also present and the dichotomization of fibres in nerve trunks which is suggestive of *collateral regeneration* (Nageotte, 1932) is occasionally seen (Pl. 1, fig. 10 and Pl. 5, figs. 90, 91). Degenerative changes are, however, most common among the fibres of the cutaneous nerve plexus where all stages in axonic disintegration can be detected (Pl. 5, figs. 86–89); on the other hand *digestive chamber* formation is less common in the plexus than after nerve section. The number of degenerating fibres in the dorsum of the ear varied greatly from one animal to another so that it is not possible to give a percentage estimate: however, in one preparation about one-quarter of the number of fibres composing the plexus were showing some signs of degeneration.

Nerve fibres undergoing degenerative changes in the cutaneous nerve plexus definitely exceed those undergoing the usual regenerative changes which occur after complete interruption of a nerve fibre; this seems to suggest that in a number of fibres these degenerative changes are transient only, and that recovery can occur without complete destruction of the fibre. The work of Speidel (1935), which has already been referred to, confirms this conclusion, for he states that early irritative changes shown by a fibre which will later recover are essentially similar to those at the beginning of Wallerian degeneration. His further work (1936), on alcoholic neuritis and recovery, again confirms these conclusions, as do the findings of Clark (1914) on regeneration of medullated nerves in the absence of embryonic nerve fibres, following non-traumatic degeneration.

It is clear, therefore, that in the ear of the rabbit cutaneous nerve fibres, more particularly of the cutaneous nerve plexus, are in a state of flux, many fibres undergoing a continuous cycle of degenerative and regenerative changes. This might perhaps have been anticipated, for the skin itself is continuously growing, quite apart from the constant trauma to which it is subjected in the course of the ordinary activities of daily life. Clark & Clark (1932) have shown that the pattern of small blood vessels in the ear of the rabbit is also in a constant state of flux; thus the nerves which supply these vessels presumably also require continual readjustment. Such eminent workers as Mayer (1881) and Bielschowsky (1935) had previously stated that the peripheral nervous system of man cannot be considered as stable and that degenerative and regenerative changes are constantly taking place. This conception of the peripheral nervous system was also accepted by Nageotte (1932) who states that collateral regeneration is a particular expression of such a process.

In order to determine whether the degenerative and regenerative changes seen in the skin from the ear of the rabbit have their counterpart in man, some pieces of human skin were examined from the point of view of their sensibility and then stained intravitally with methylene blue and removed. It was

observed that over the pressure point of the elbow in a number of individuals it is possible, by careful examination with a V. Frey hair and a fine sharp needle, to demonstrate a small area of anaesthesia and analgesia. Accordingly a piece of skin from this region, which included both areas in which the sensibility was normal and in which it was absent, was stained and removed.

The skin contained a large number of degenerating fibres, particularly in the cutaneous nerve plexus. In the affected area a number of isolated nerve nets were seen immediately beneath the epithelium, in which the individual beads were greatly enlarged and no longer interconnected. The collections of enlarged beads in the dermal papillae were at first mistaken for isolated complex end-organs until the whole area was examined and their true nature became clear. In the normal zone the nerve nets were seen to spring from the cutaneous nerve plexus and the individual beads were fine and interconnected. A number of regenerating fibres were also seen in the cutaneous nerve plexus of the adjacent normal zone. Thus it appears that in this area of skin, which is particularly exposed to minor degrees of pressure trauma, the nerve fibres of the cutaneous plexus show evidence of various degrees of degeneration and regeneration. Histological observations were also made on a small piece of skin removed from the flexor aspect of the forearm, in which sensibility was found to be normal. Even here, a small number of degenerating fibres were found to be present in the cutaneous nerve plexus.

Finally an opportunity arose of examining skin from the pad of a finger which had to be amputated because of Dupuytren's contracture. This contracture, it should be noted, had bound the finger down towards the palm from which position it could not be extended, so that for some time it had not been in use and had been preserved from external pressure. Sensibility over the pad was normal and similar to that over the opposite finger. No degenerating or regenerating fibres were seen in the area of skin examined.

Although the number of cases studied histologically is small, these findings suggest that in man there is also a process of nerve degeneration and regeneration continuously taking place in the skin, particularly among the fibres of the cutaneous nerve plexus; it also appears that in areas which are particularly subject to minor traumata the amount of such a change is at a maximum, whereas in a finger pad which was almost completely protected from trauma no degenerating fibres were seen. It is suggested that these findings may explain to some extent the varying and puzzling results which commonly follow refined tests of skin sensibility in normal individuals and in cases of peripheral nerve injuries. Further, the apparent lability of the fibres of the cutaneous nerve plexus may have an important bearing on the pattern of cutaneous regeneration and the shrinkage of the area of sensory loss which on occasion rapidly follows peripheral nerve section.

The fact that a number of degenerating and regenerating fibres can normally be found in peripheral nerve trunks (Bielschowsky, 1935) must obviously be taken into account when basing conclusions upon findings of not more than

1–5 % degenerating fibres in nerve trunks after experimental operative procedures.

SUMMARY

1. An account of the early stages in Wallerian degeneration which follow nerve section in the skin of the dorsum of the rabbit's ear has been given. The results of methylene-blue staining, silver impregnation, and specific staining for myelin have been compared.

2. The Schwann cells in the skin of the dorsum of the rabbit's ear show no reaction to vital dyes for 336 hr. after nerve section.

3. In the skin from the dorsum of the ear of normal unoperated rabbits a small proportion of the nerve fibres show evidence of either degenerative or regenerative changes. Similar changes have been seen in a piece of skin from the elbow region of a human subject. These changes are most marked in the cutaneous nerve plexus.

The cost of this investigation was defrayed by grants made to one of us (G. W.) by the Medical Research Council. The other author (P. G.) wishes gratefully to acknowledge a grant made by the Nuffield Committee for the Advancement of Medicine.

REFERENCES

Bielschowsky, M. (1935). *Handbuch d. Neurologie*, O. Bumke and O. Foerster, 2nd ed. **1**. Anatomie Berlin: J. Springer.

Cajal, S. R. (1928). *Degeneration and Regeneration of the Nervous System*, **1**. Oxford University Press.

Clark, E. (1914). *J. comp. Neurol.* **24**, 61.

Clark, E. R. & Clark, E. L. (1932). *Amer. J. Anat.* **49**, 441.

Duncan, D. (1934). *J. comp. Neurol.* **60**, 437.

Marinesco, G. (1907). La nature intime du processus de dégénérescence des Nerfs. *Pr. méd.* **14**, 105.

Mayer, S. (1881). *Z. Heilk.* **2**, 154.

Nageotte, J. (1932). Sheaths of the peripheral nerves. Nerve degeneration and regeneration. Section V in *Cytology and Cellular Pathology of the Nervous System*, **1**, 189–239. W. Penfield, editor. New York: P. B. Hoeber.

Speidel, C. C. (1933). *Amer. J. Anat.* **52**, 1.

—— (1935). *J. comp. Neurol.* **61**, 1.

—— (1936). *J. comp. Neurol.* **64**, 77.

Weddell, G. (1941). *J. Anat., Lond.*, **75**, 346.

Weddell, G. & Harpmann, J. A. (1940). *J. Neurol. Psychiat.* **3**, 319.

Weddell, G., Harpmann, J. A., Lambley, D. G. & Young, L. (1940). *J. Anat., Lond.*, **74**, 255.

Woollard, H. H. (1924). *J. Anat., Lond.*, **58**, 89.

Woollard, the late H. H., Weddell, G. & Harmann, J. A. (1940). *J. Anat., Lond.*, **74**, 413.

APPENDIX

Photographic Atlas showing the normal structure and early stages in the Wallerian Degeneration of cutaneous nerve fibres in the ear of the rabbit.

The photomicrographs reproduced on Pls. 1–5 were all taken on a 35 mm. film at standard magnifications. The individual prints were enlarged to sizes suitable for reproduction purposes and the magnifications accurately determined. The diameters of many of the nerve fibres can be directly compared, others can be compared after making suitable allowances for differences in magnifications.

Plate 1

All the photomicrographs reproduced on this plate are of normal cutaneous nerve fibres. The results of silver impregnation (figs. 1–7), methylene-blue staining (figs. 8–13) and specific staining for myelin (figs. 14–18) are shown. Details of normal cutaneous nerve fibres are described on pp. 67–9 of the text.

Fig. 1 is from a silver preparation (× 820) showing the structure of the axis cylinders of large myelinated fibres in nerve trunks. Only axis cylinders and Schwann nuclei have been impregnated; there is a suggestion of fibrillation near the node of Ranvier, but this is not seen along the course of the fibre elsewhere. A Schwann nucleus, slightly out of focus, can be seen indenting the axis cylinder of the left-hand fibre. The soldering disc of Ranvier is clearly seen at a nodal point along the course of the right-hand fibre.

Fig. 2 is also from a silver preparation (× 820) and shows the faint outline of a Schwann nucleus indenting the axis cylinder of a smaller myelinated fibre in a nerve trunk. There is some fibrillation of that part in the axis cylinder not indented by the nucleus. This fibrillation is uncommon in normal fibres.

Fig. 3 is from a silver preparation (× 600) and shows a non-medullated fibre in a nerve trunk. The Schwann nuclei are seen to lie on alternate sides of the fibre; this arrangement is maintained as the fibre is traced distally.

Fig. 4 is from a silver preparation (× 820) and shows nerve fibres of the cutaneous nerve plexus passing in three different directions. The Schwann nuclei are also impregnated. The axis cylinders show no fibrillation although the diameters vary somewhat along the course of the fibres.

Fig. 5 is from a silver preparation (× 820) in which the technique had been modified slightly, resulting in the impregnation of the Schmidt-Lantermann clefts of the myelin sheaths. The Schwann nuclei are not impregnated. The fibres illustrated are lying in nerve trunks.

Fig. 6 is from a silver preparation (× 820) in which the technique had been further modified resulting in the impregnation of the annular rings and infundibular membranes belonging to the cleft systems. The fibre illustrated is lying in a nerve trunk. The Schwann nuclei are also impregnated.

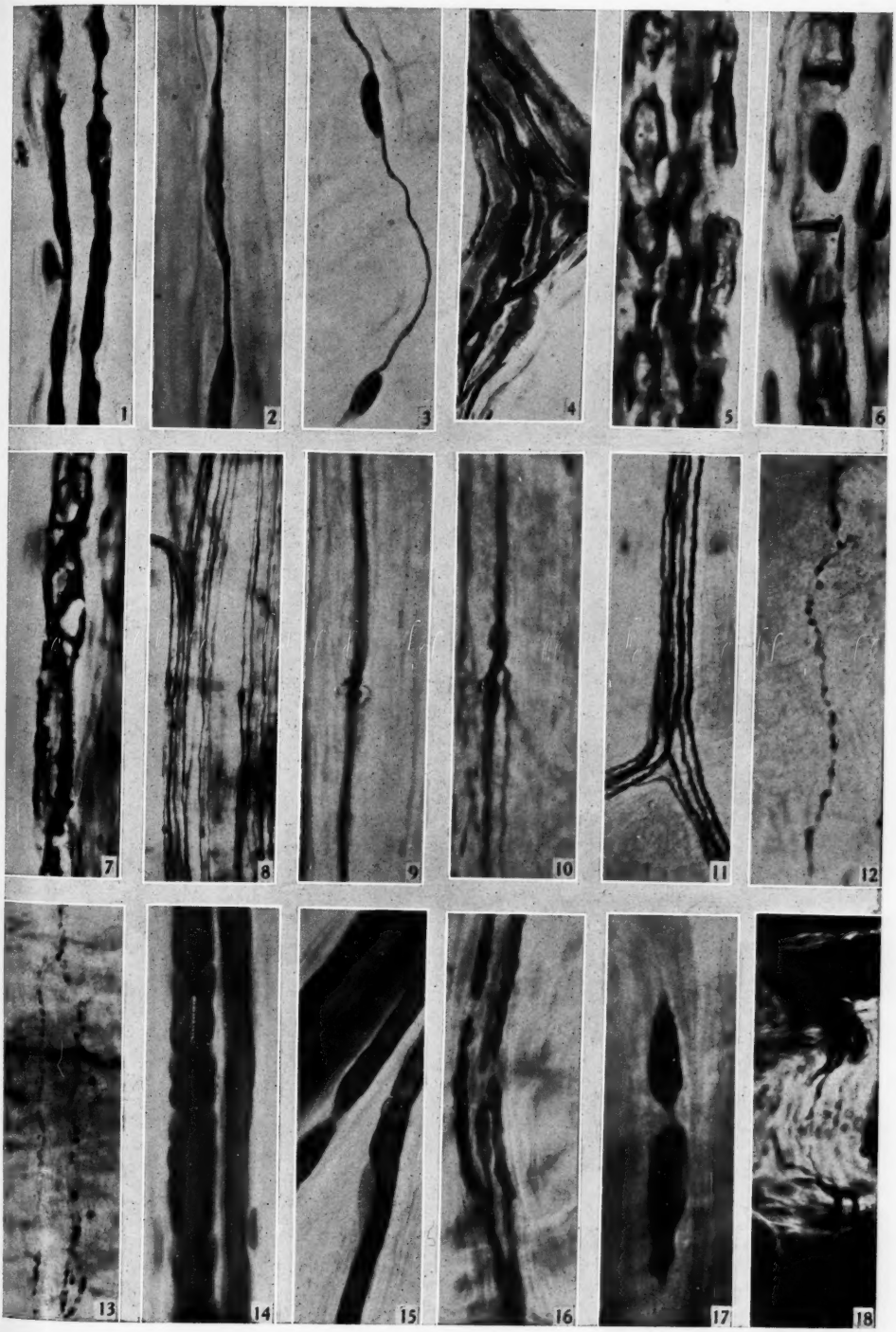

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Fig. 7 is from a silver preparation (×820) in which the impregnation methods had been still further modified. The photomicrograph shows the outline of the impregnated 'cytoplasmic veil of Schwann' (Cajal, 1928). No Schwann nuclei are seen. The fibre is lying in a nerve trunk.

Fig. 8 is from a methylene-blue preparation (×125) and shows a subcutaneous nerve trunk consisting of a variety of fibre sizes. A number of the fibres are seen leaving the trunk. There is no dichtomization but a reduction in the number of fibres in the trunk which proceeds distally.

Fig. 9 is from a methylene-blue preparation (×555) and shows a node of Ranvier along the course of a single large medullated fibre. The soldering disc of Ranvier is clearly seen and also the double bracelets of Nageotte. There is also a faint outlining of the myelin sheath close to the node.

Fig. 10 is from a methylene-blue preparation (×555) and shows a large medullated fibre giving rise to a smaller medullated fibre at a nodal point. The two fibres were traced to the cutaneous nerve plexus where they pursued remarkably similar courses. A small sprout which ends abruptly can be seen arising from the opposite side of the node.

Fig. 11 is from a methylene-blue preparation (×277) showing fibres of the cutaneous nerve plexus. Some of the fibres are dichotomizing.

Fig. 12 is from a methylene-blue preparation (×1250) showing a fine beaded terminal from a subepidermal nerve net, ending between the cells of the deep layer of the epidermis. The beads are interconnected. The outline of the epithelial cells can be clearly seen in the same focal plane as the nerve fibre.

Fig. 13 is from a methylene-blue preparation (×1250) showing portions of a nerve net ending around an artery. The transverse striations in the background are smooth muscle cells. The beads are all interconnected although this does not appear to be strictly true in the photomicrograph owing to the varying focal planes in which the fibres lie.

Fig. 14 is from a preparation stained for myelin (×833). The fibres are lying in nerve trunks. The larger fibre possesses many Schmidt-Lantermann clefts, while the smaller fibre possesses few. Schwann nuclei can be seen outside the myelin sheath of both fibres.

Fig. 15 is also from a preparation stained for myelin (×833). The fibres are of smaller diameter than those shown in the previous figure. A node of Ranvier is clearly seen and a Schwann nucleus strongly indenting the wall of the sheath. No Schmidt-Lantermann clefts are visible.

Fig. 16 is from a preparation stained for myelin (×555) showing the myelin sheaths surrounding fibres of the cutaneous nerve plexus. There are gaps between the segments of the sheaths but these do not correspond morphologically to nodes of Ranvier. No Schmidt-Lantermann clefts are seen.

Fig. 17 is from a preparation stained for myelin (×1250) showing two small isolated segments along the course of a fibre on its way to a hair. The fibre was completely medullated while in the cutaneous nerve plexus, but lost this sheath soon after leaving; it approached the hair as a non-medullated nerve fibre save for the short segments illustrated which were isolated at some distance from the main sheath.

Fig. 18 is from a preparation stained for myelin (×357) showing the distance to which the medullary sheath may surround nerve fibres supplying hair follicles. In this instance the sheaths extend as far as the terminal ramifications of the nerve on the follicle itself.

Plate 2

The photomicrographs, figs. 19–28, reproduced on this plate show the condition of cutaneous nerve fibres 12 hr. after section of the nerve trunk. Figs. 29–36 show the conditions 24 hr. after nerve section. Descriptions of these changes are given on pp. 69–71 and p. 72 respectively of the text.

Fig. 19 is from a methylene-blue preparation (×555) showing on the left side commencing fibrillation of a medium-sized medullated fibre close to a node of Ranvier. A non-medullated fibre on the right shows segmentation of the axis cylinder with gaps between the segments in a number of places.

Fig. 20 is from a methylene-blue preparation (×555) showing two small medullated fibres in a nerve trunk. The left-hand fibre shows a granular fragmentation of its axis cylinder; the right-hand fibre shows marked irregularity of the axis cylinder but fragmentation has not yet commenced.

Fig. 21 is from a silver preparation (×820) showing a medium-sized medullated fibre in a nerve trunk. At the point depicted, the axis cylinder is undergoing a considerable degree of fibrillation. Such fibrillated swellings were seen at frequent intervals along the course of the fibre.

Fig. 22 is from a methylene-blue preparation (×555) showing the outline of the medullary sheath of a large fibre in a nerve trunk. The sheath contains a network of blue beaded threads. The outline of two annular rings can also be seen. This fibre may have undergone precocious degeneration.

Fig. 23 is from a methylene-blue preparation (×555) and is very similar to fig. 22 except that the outline of the sheath is more diffusely stained and the network in the upper part is unbeaded. Two annular rings can be seen in the lower half and a large diffusely stained blue mass is seen in the position of a node along the course of the fibre. This fibre also may have undergone precocious degeneration.

Fig. 24 is from a silver preparation (×600), stained to show annular rings and infundibular membranes belonging to the cleft systems. These are now seen to be irregular.

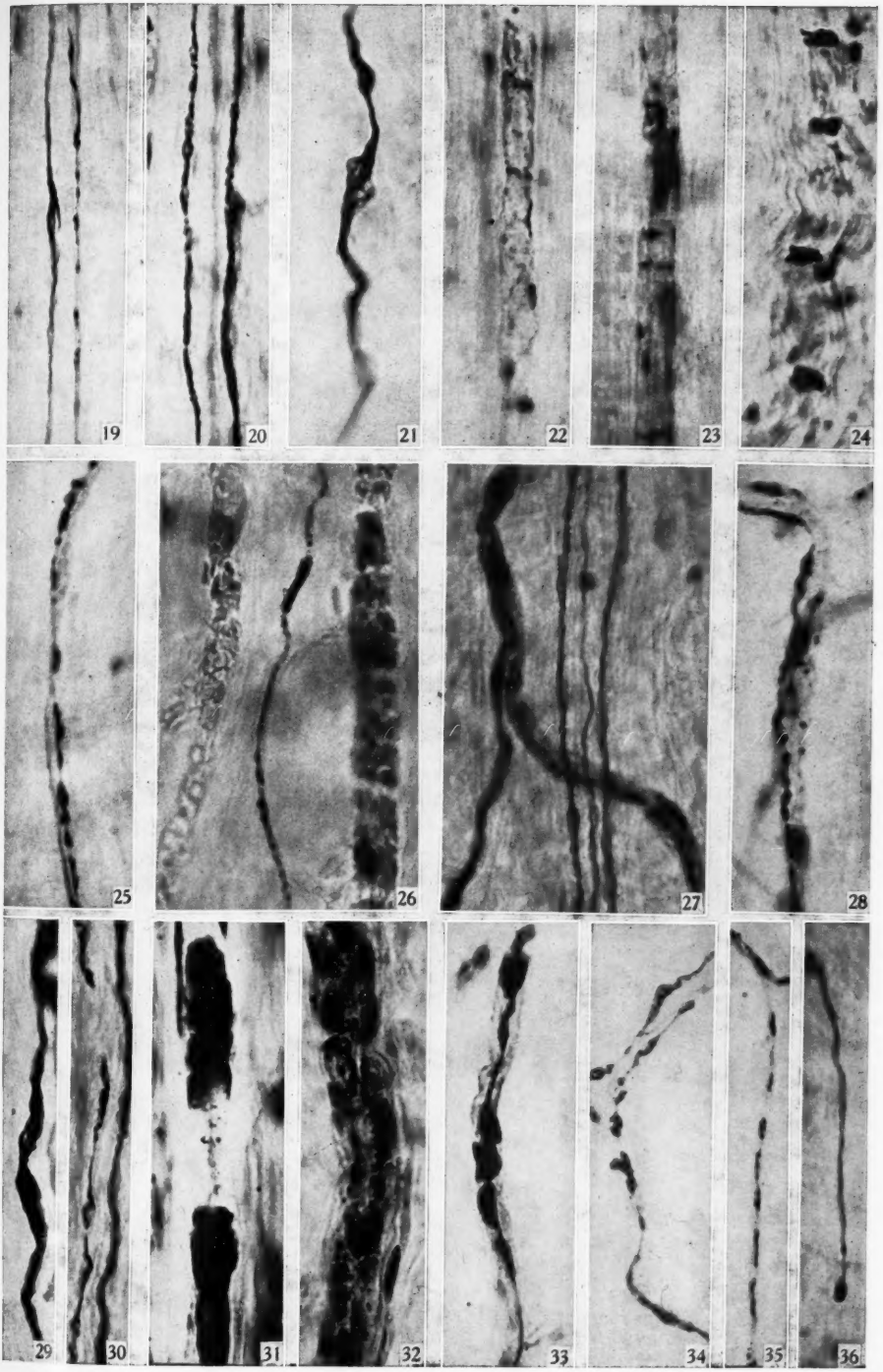

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Fig. 25 is from a methylene-blue preparation (× 555) showing axis cylinder fragmentation in a fibre of the cutaneous nerve plexus.

Fig. 26 is from a methylene-blue preparation (× 555) showing granular fragmentation of a vasomotor fibre; the Schwann sheath is faintly outlined and its nucleus is darkly stained.

Fig. 27 is from a methylene-blue preparation (× 555) showing fragmentation of the axis cylinders of vasomotor fibres contained within a single Schwann sheath. The outline of the sheath is clearly visible, and the Schwann nuclei are more darkly stained. On a slightly deeper plane are three small medullated fibres in which the axis cylinders are undergoing a minimal degree of fibrillation. The Schwann nuclei and sheaths of these fibres are not stained.

Fig. 28 is from a methylene-blue preparation (× 555) showing irregular swellings along the course of a fibre of the cutaneous nerve plexus.

Fig. 29 is from a methylene-blue preparation (× 520) showing an increase in the axonic swelling and fibrillation close to a node of Ranvier of a large medullated fibre in a nerve trunk. The changes at the node itself are minimal.

Fig. 30 is from a methylene-blue preparation (× 520) showing a further stage in fragmentation of the axis cylinder of a small medullated fibre in a nerve trunk. The fibre on the right of the picture is of the small medullated variety but shows minimal changes only consisting of a slight swelling of the axis cylinder and blurring of its outline.

Fig. 31 is from a silver preparation (× 700) showing a precociously degenerating large medullated fibre in a nerve trunk. There has been a complete granular disintegration of the axis cylinder and the myelin sheath is showing *ellipsoid* formation. The Schmidt-Lantermann cleft outlines can be seen and the *ellipsoids* appear to be forming by fracture at these clefts. A few axonic remains are seen connecting the *ellipsoids*.

Fig. 32 is from a silver preparation (×700) showing another precociously degenerating fibre in a nerve trunk in which there is vacuolation and granular disintegration of the axis cylinder. No *ellipsoid* formation can be seen. It is possible that some of the larger darkly stained elements may be myelin droplets. The outline of the Schwann sheath can be seen together with a nucleus which appears normal.

Fig. 33 is from a silver preparation (× 700) showing irregular swellings along the course of the axis cylinder of a small medullated fibre in a nerve trunk. The outline of the myelin sheath can be clearly seen and is commencing to form *ellipsoids*. This photomicrograph should be compared with the methylene-blue preparation of a small medullated fibre in the cutaneous nerve plexus (fig. 35).

Fig. 34 is from a methylene-blue preparation (× 400) showing granular disintegration of the axis cylinders of fibres in the cutaneous nerve plexus.

Fig. 35 is from a methylene-blue preparation (×221) showing irregular swelling and segmentation of the axis cylinder of a small medullated fibre in the cutaneous nerve plexus. The faint outline of the myelin sheath undergoing *ellipsoid* formation is seen.

Fig. 36 is from a methylene-blue preparation (×520) showing a non-medullated fibre on its way from the cutaneous nerve plexus to a subepidermal nerve net. It ends in a granular blob just before reaching the net; the fibres of the latter appear to be normal.

Plate 3

The photomicrographs, figs. 37–47, reproduced on this plate show the condition of cutaneous nerve fibres 48 hr. after section of their nerve trunks. Figs. 48–55 are from a series of photomicrographs taken at different positions along the course of the same large medullated fibre in a nerve trunk showing the condition of the fibre 96 hr. after interruption of its central connexion. Fig. 48 is towards the base of the ear, and the subsequent figures show the changes which occur when the fibre is traced for approximately 1½ cm. distally. Fig. 56 shows the condition of a non-myelinated fibre in the same trunk. Descriptions of these changes are given on pp. 72 and 73 and 73 and 74 of the text respectively.

Fig. 37 is from a methylene-blue preparation (×555) showing a form of granular disintegration of the axis cylinder of a large medullated fibre in a nerve trunk. The axis cylinder is swollen and its outline is indistinct; the myelin sheath is stained and in addition contains a number of granules. Two distorted annular rings or infundibular membranes can be seen.

Fig. 38 is from a methylene-blue preparation (×555) showing two large medullated fibres lying side by side in a nerve trunk. In the left-hand fibre the axis cylinder has completely disintegrated. Dark particles can be seen in the diffusely stained myelin sheath. The remains of the axis cylinder are most clearly seen at the node of Ranvier. The right-hand fibre shows a marked swelling of the axis cylinder which has an indistinct outline. The myelin sheath is faintly stained, and its outline can just be seen in the photograph.

Fig. 39 is from a methylene-blue preparation (×555) showing the axis cylinder of a large myelinated fibre in a nerve trunk. It has undergone much fibrillation and some slight granular fragmentation close to the nodal point. The soldering disc of Ranvier is not apparent at the node but the reinforced *Innenscheide* of Mönckeberg and Bethe is clearly seen.

Fig. 40 is from a methylene-blue preparation (×555) showing the axis cylinder of a large myelinated fibre in a nerve trunk. It has undergone marked fibrillary changes. The soldering disc of Ranvier and double bracelets of Nageotte are seen at the nodal point.

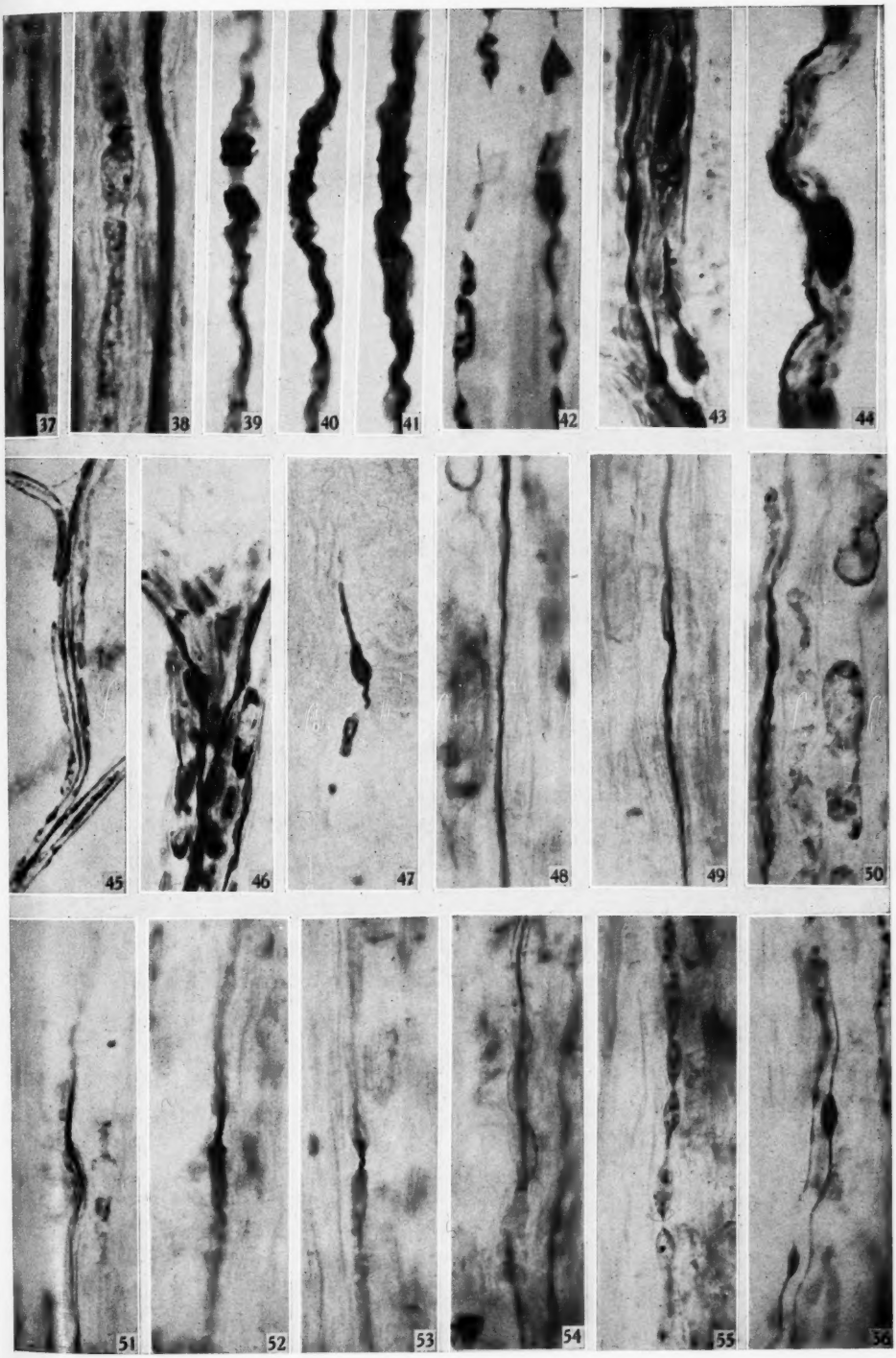

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Fig. 41 is from a methylene-blue preparation (× 555). The changes resemble those seen in fig. 40 with the exception that the fibre is of larger diameter and the double bracelets of Nageotte are clearly spinous. The spinous processes are thin and not very darkly stained; they are best seen on the right side. The fibre is lying in a nerve trunk.

Fig. 42 is from a methylene-blue preparation (× 555) showing fragmentation, with retraction of the axis cylinder remnants, of smaller medullated fibres in nerve trunks. The remains are lying in faintly stained myelin *digestive chambers*.

Fig. 43 is from a silver preparation (× 1000). On the right, axis cylinder remains of a small myelinated fibre are seen within *digestive chambers*. On the left, the axis cylinder is considerably swollen at irregular intervals along its course but no other changes are visible. The fibres are lying in a nerve trunk. (Compare fig. 42.)

Fig. 44 is from a methylene-blue preparation (× 1250) showing two non-medullated fibres within a single neurilemma sheath. The axis cylinder on the right has completely disintegrated whilst that of the left-hand fibre has just commenced to fragment. The Schwann nucleus is deeply stained.

Fig. 45 is from a methylene-blue preparation (× 200) showing the formation of *digestive chambers* among fibres of the cutaneous nerve plexus. A number of the fibres show only a fibrillation of the axis cylinder, but when traced further towards their destinations these same axis cylinders undergo fragmentation and *ellipsoid* formation.

Fig. 46 is from a silver preparation (× 350) which also shows the formation of *digestive chambers* along the course of medullated fibres of the cutaneous nerve plexus. A number of the larger fibres again only show fibrillation of the axis cylinder. Figs. 45 and 46 are strictly comparable.

Fig. 47 is from a methylene blue preparation (× 1250). It shows the considerable swelling and retraction of the beaded terminals of the cutaneous nerve net which takes place at this stage; the beads are no longer interconnected. This figure should be compared with Pl. 1, fig. 12, which is from a normal preparation at the same magnification.

Figs. 48–55 are from methylene-blue preparations (× 500) showing the changes which occur along the course of a large medullated fibre in a nerve trunk when traced distally towards its termination.

Fig. 48. The axis cylinder shows a minimal degree of fibrillation and the myelin sheath is only just visible.

Fig. 49. There is an increase in the degree of fibrillation of the axis cylinder particularly in the neighbourhood of the nodal point. Outlines of reinforced *Innenscheide* are clearly seen close to the node.

Fig. 50. There is a further increase in the degree of axis cylinder fibrillation. The outline of the myelin sheath can now be seen clearly. There is still no fibrillation at the nodal point. Immediately to the right of the fibre myelin *digestive chambers* can be seen containing twisted axis cylinder remnants.

Fig. 51 shows a further stage in the disintegration of the axis cylinder. The myelin sheath is more clearly outlined than in the previous figure. There are still no changes at the nodal point.

Fig. 52 shows complete outlining of the myelin sheath and commencing *ellipsoid* formation close to the nodal point.

Fig. 53 shows a further stage in *ellipsoid* formation. The axis cylinder is still intact at the nodal point.

Fig. 54 shows myelin *ellipsoids* surrounding axis cylinder remnants. The *ellipsoids* are interconnected except at the nodal point where changes are, however, now apparent. The axis cylinder can be seen to fragment as it is traced distally and forms the bodies of Elzholz in the *digestive chambers*.

Fig. 55 shows pieces of disintegrated axis cylinder within myelin *ellipsoids* which are beginning to separate. Nodal points can no longer be recognized.

Fig. 56 is from a methylene-blue preparation (× 555) showing two resistant non-medullated fibres. The axis cylinder splits to enclose a fusiform swelling along the course of each fibre.

Plate 4

The photomicrographs, figs. 57–68, reproduced on this plate show the condition of cutaneous nerve fibres 96 hr. after section of the nerve trunk; figs. 69–73 show the condition 156 hr. after nerve section. Description of these changes are given on pp. 73 and 74 and 74 and 75 of the text respectively.

Fig. 57 is from a methylene-blue preparation (× 555) showing the condition of the non-medullated fibre depicted in Pl. 3, fig. 56, when traced distally towards the cutaneous nerve plexus.

Fig. 58 is from a silver preparation (× 820) showing myelin *ellipsoids* containing axis cylinder remnants lying in nerve trunks. The Schwann nuclei are somewhat enlarged.

Fig. 59 is from a silver preparation (× 820) showing myelin *ellipsoids* and portions of granular fragmented axis cylinders among the fibres of the cutaneous nerve plexus.

Fig. 60 is from a methylene-blue preparation (× 125) showing the reduction in the number of axonic remains in the cutaneous nerve plexus. (Compare Pl. 1, figs. 4, 11.)

Fig. 61 is from a silver preparation (× 820) showing a granular fragmented axone within an impregnated neurilemma sheath among fibres of the cutaneous nerve plexus. (Compare Pl. 3, fig. 44.)

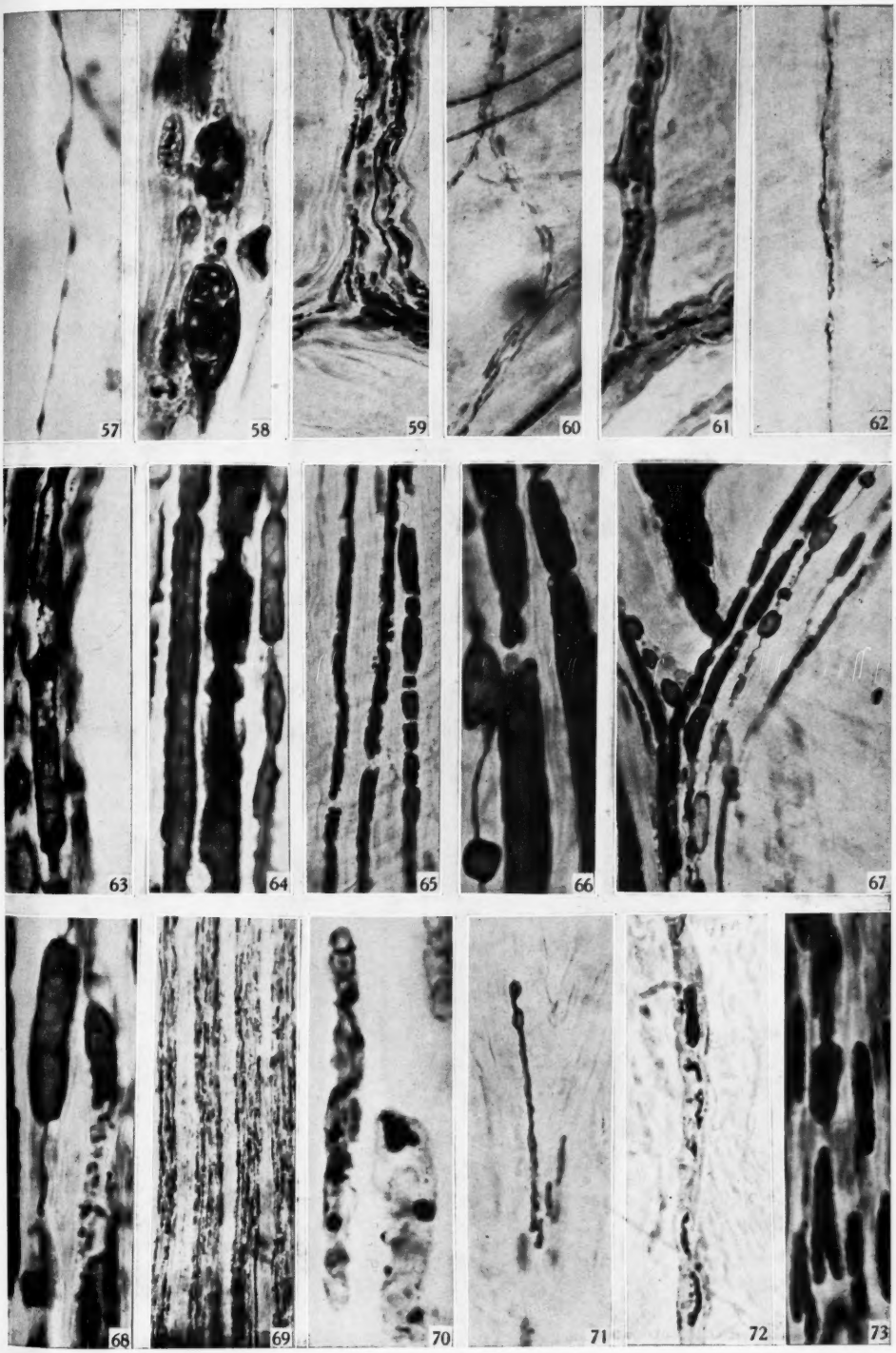

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Fig. 62 is from a silver preparation (× 850) showing a fragmented axone within a faintly outlined neurilemma sheath. (Compare Pl. 3, fig. 44.)

Fig. 63 is from a silver preparation (× 820) in which the technique had been modified to impregnate the Schmidt-Lantermann clefts. The outline of the myelin sheath and Schmidt-Lantermann clefts of a fibre in a nerve trunk are seen. *Ellipsoid* formation is commencing. The sheath appears to be fragmenting at a Schmitt-Lantermann cleft.

Fig. 64 is from a preparation stained for myelin (× 820) showing the myelin sheaths of large fibres in a nerve trunk undergoing dislocation and *ellipsoid* formation. The point of fracture is commonly at a Schmidt-Lantermann cleft.

Fig. 65 is from a preparation stained for myelin (× 350) showing the condition of the myelin sheath of smaller fibres in the nerve trunk.

Fig. 66 (× 820), fig. 67 (× 350) and fig. 68 (× 820) are from a preparation stained for myelin showing the condition of myelinated fibres in the cutaneous nerve plexus. Myelin *ellipsoids* are seen to be interconnected by myelin threads in a number of cases.

Fig. 69 is from a methylene-blue preparation (× 125) showing the condition of the fibres in a main nerve trunk 168 hr. after nerve section. All the nerves are seen to be undergoing degenerative changes and the majority of them are in the form of *digestive chambers* containing axis cylinder remnants.

Fig. 70 is from a methylene-blue preparation (× 950) showing a *digestive chamber* containing small irregular axis cylinder remnants, together with a twisted axis cylinder segment.

Fig. 71 is from a methylene-blue preparation (× 555) showing an axis cylinder remnant passing up from a nerve trunk towards the cutaneous nerve plexus. No remnants could be seen further distally from this point. The faint outline of the connective tissue sheath surrounding the branch bundle can be seen.

Fig. 72 is from a silver preparation (× 820) showing granular axis cylinder remnants within a faintly outlined neurilemma sheath in a fibre lying in a bundle which has just branched off the main trunk. When traced distally no further axis cylinder remnants could be seen.

Fig. 73 is from a silver preparation (× 820) showing a large number of Schwann nuclei within a continuous sheath lying in the position of a small nerve bundle. The nuclei are larger and more elongated than normal and contain numerous dark granules. (Compare fig. 58.)

Plate 5

The photomicrographs, figs. 74–76, reproduced on this plate show the condition of cutaneous nerve fibres 336 hr. after section of the nerve trunk; figs. 77–81 are from *intermediate zones* and figs. 82–91 show stages in the degeneration and regeneration of nerve fibres in the skin of normal unoperated rabbits. Descriptions of these changes are given on pp. 75, 76 and 77 and 80 and 81 respectively of the text.

Fig. 74 is from a methylene-blue preparation (×277) showing a large decrease in the number of fibres containing axis cylinder remnants in a nerve trunk. All remnants are within *digestive chambers*.

Fig. 75 is from a methylene-blue preparation (×1250) showing a twisted axis cylinder remnant within a myelin *ellipsoid*.

Fig. 76 is from a silver preparation (×820) showing a number of Schwann nuclei and the neurilemma sheath along the original course of a bundle of nerve fibres of the cutaneous nerve plexus. As compared with Pl. 4, fig. 73, the nuclei are smaller and the sheath shrunken and apparently fibrillated.

Fig. 77 is from a methylene-blue preparation (×125) of an *intermediate zone*. Both degenerating and normal nerve fibres are seen running together at the level of the nerve trunks.

Fig. 78 is from a methylene-blue preparation (×555) showing a stained nucleated, slightly fibrillated Schwann sheath. It contains what appear to be axis cylinder remnants along its axial line in a few places but the greater part of the sheath is empty. Such sheaths do not stain in completely denervated zones.

Fig. 79 is from a methylene-blue preparation (×555) showing a stained sheath in which there are no axis cylinder remnants but a number of darkly stained particles in addition to Schwann nuclei and some unstained spherical droplets presumably of degenerating myelin.

Fig. 80 is from a methylene-blue preparation (×555) showing a number of apparently normal non-medullated axis cylinders within a stained slightly fibrillated Schwann sheath. One fibre ends abruptly on entering a side branch of the sheath which is more clearly fibrillated. This abrupt termination of the axis cylinder is suggestive of a growth cone.

Fig. 81 is from a methylene-blue preparation (×555) showing the club-shaped termination of an axis cylinder which arose from an unmyelinated fibre of the cutaneous nerve plexus and passed into the surrounding tissues of an *intermediate zone* on the leg of a rabbit.

Fig. 82 is from a silver preparation (×820) showing a degenerating nerve fibre within a nerve trunk from the skin of a normal unoperated ear. The fibre in question is on the extreme right of the photograph and is at the stage of *digestive chamber* formation. (Compare Pl. 4, fig. 58.)

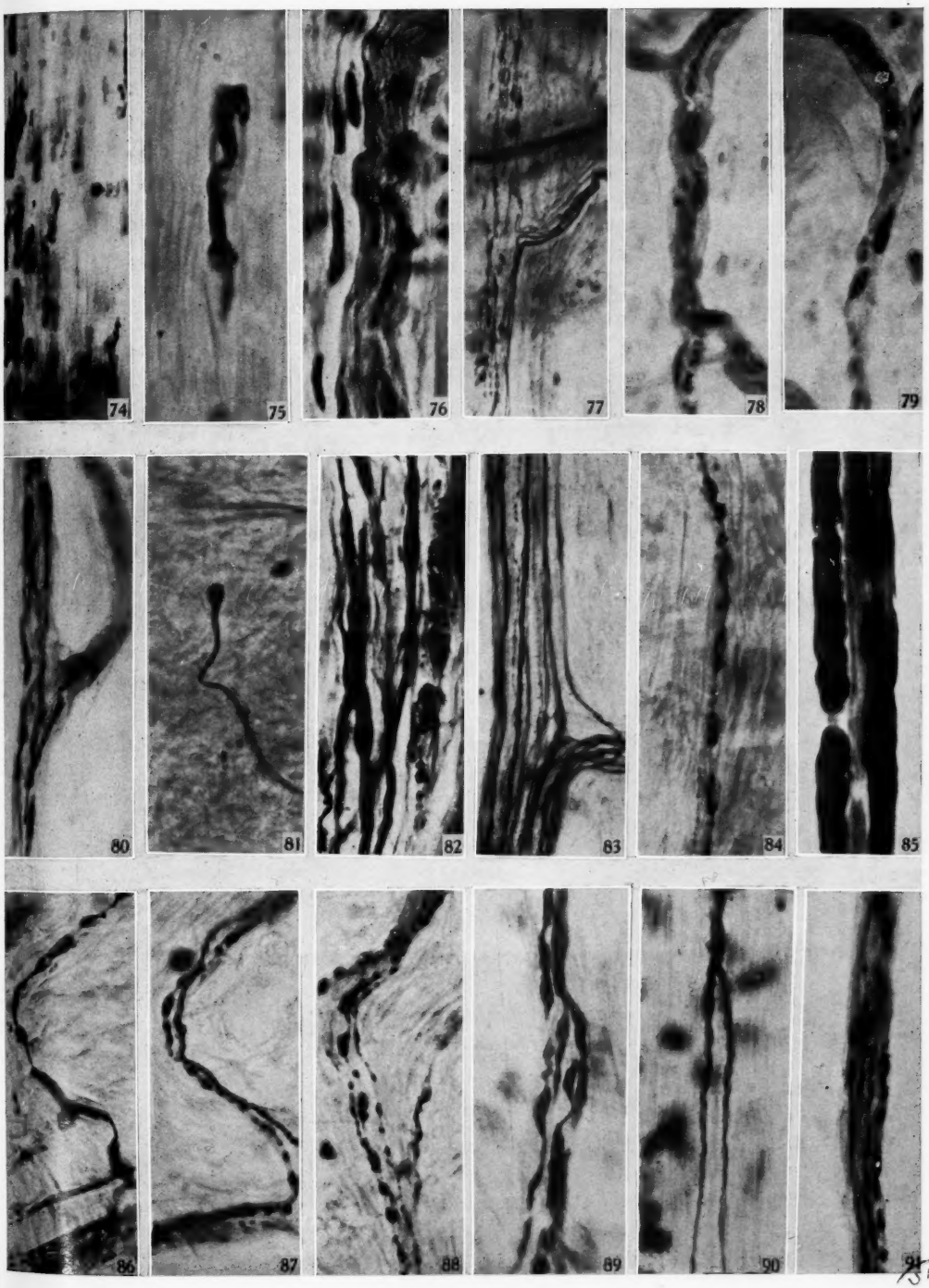
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Fig. 83 is from a methylene-blue preparation (× 277) showing a degenerating non-myelinated fibre lying among normal fibres of a nerve trunk. (Compare Pl. 4, fig. 57.)

Fig. 84 is from a methylene-blue preparation (× 555) showing a small medullated fibre in a nerve trunk undergoing degeneration. The fibre is at the stage of digestive chamber formation. (Compare Pl. 3, fig. 52.)

Fig. 85 is from a preparation stained for myelin (× 833) showing two large medullated fibres in a nerve trunk. The contour of the right-hand fibre is smooth, the contour of the left-hand fibre, however, is irregular. (Compare Pl. 4, fig. 64.)

Fig. 86 is from a methylene-blue preparation (× 555) showing a small medullated fibre at the stage of commencing *digestive chamber* formation. The fibre has just left the cutaneous nerve plexus and is on its way to a hair follicle. (Compare Pl. 3, fig. 52.)

Fig. 87 is from a methylene-blue preparation (× 555) showing a normal and a degenerating nerve fibre lying side by side in the cutaneous nerve plexus. (Compare Pl. 1, figs. 4, 11.)

Fig. 88 is from a methylene-blue preparation (× 555) showing a number of degenerating fibres in the cutaneous nerve plexus. (Compare Pl. 1, figs. 4, 11.)

Fig. 89 is from a preparation stained for myelin (× 555). The sheaths surrounding fibres in the cutaneous nerve plexus are markedly irregular. (Compare Pl. 1, fig. 16.)

Fig. 90 is from a methylene-blue preparation (× 555) and is another example of *collateral regeneration* in a nerve trunk. (Compare Pl. 1, fig. 10.)

Fig. 91 is from a methylene-blue preparation (× 555) showing a stained nucleated Schwann sheath in the cutaneous nerve plexus containing degenerating, regenerating, and normal nerve fibres. The outline of the normal fibre can be clearly seen in the lower part of the photograph but passes into a slightly deeper plane as it is traced upwards and then goes out of focus to a great extent.

VARIATIONS IN ORGAN SIZE CAUSED BY CHRONIC TREATMENT WITH ADRENAL CORTICAL COMPOUNDS

AN EXAMPLE OF A DISSOCIATED ADAPTATION TO A HORMONE

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It is well established that, after continued treatment, the animal organism may lose its sensitivity to certain hormone preparations. Recently it has been shown (Selye, 1940*a*) that adaptation can be acquired even to pure crystalline steroid hormones. This fact was of particular interest since pure steroids do not lead to any serological immunity or 'anti-hormone' formation. It has been emphasized that the adaptation to steroid hormones is not entirely specific. For instance, animals rendered resistant to naturally occurring oestrogens also show some resistance to artificial oestrogens such as stilboestrol. Furthermore, pretreatment with progesterone may cause some resistance to adrenal cortical compounds and vice versa (Selye, 1941*a*). As a counterpart to these observations, it may be stated that adaptation to a certain effect of a steroid hormone does not necessarily imply that the sensitivity of the organism to all other actions of this compound is likewise diminished or abolished. This was first shown by the observation that the enlargement of the pituitary, the adrenal cortex, and the corpus luteum, which is elicited in the rat by oestrogen overdosage, regresses after several weeks of treatment in spite of continued daily oestrogen administration although the responsiveness of the uterus and vagina does not appear to be diminished (Selye *et al.* 1935). Even the loss of sensitivity of those organs which eventually become resistant to stimulation by oestrogens does not always appear at the same time. Thus in the above-mentioned experiments, it was found that the enlargement of the corpora lutea vanishes with great regularity within a short period, while that of the adrenals and of the pituitary persists much longer. In fact, some animals may develop tumour-like pituitary enlargements even though their ovaries, and in some cases their adrenals, have become irresponsive.

This communication deals with experiments in which rats were chronically treated with desoxycorticosterone acetate and it was found that, although the hormone invariably produces thymus and spleen atrophy in short term experiments, no such atrophy is seen in the case of prolonged treatment. Indeed, in many instances, the thymus and spleen of desoxycorticosterone acetate treated rats were larger than those of untreated controls. However, in spite of this acquired resistance to its thymus and spleen atrophy-producing action, the compound retains most of its other physiological and morphogenetic effects.

The fact that active purified adrenal cortical extracts produce thymus atrophy when given in sufficiently large doses was shown some time ago in experiments on the rat (Selye, 1936) and since confirmed by Carriere *et al.* (1937). It is not surprising that Low (1938) obtained entirely negative results, since he administered insufficient quantities of cortical extracts. Wells & Kendall (1940*a*, *b*) and Kendall (1940) claimed that some adrenal cortical steroids such as dehydrocorticosterone, corticosterone, corticosterone acetate, and C_{17}-hydroxy-dehydrocorticosterone (Kendall's compound 'E') cause pronounced thymus atrophy while desoxycorticosterone, desoxycorticosterone acetate, and an amorphous adrenal cortical fraction rich in the 'life-maintaining cortical hormone' have no such effect. However, Selye (1940*b*) showed definitely that desoxycorticosterone acetate causes marked thymus atrophy in the rat if given in sufficiently high doses over a comparatively short period. Other lymphatic organs, such as the spleen, usually also involute under the influence of adrenal cortical compounds (Selye, 1940*b*; Wells & Kendall, 1940*b*). Other constant and reliable indices of desoxycorticosterone acetate action in the intact rat are atrophy of the adrenal cortex (Selye, 1940*c*), enlargement of the kidney (Selye, 1940*b*, 1941*b*), and the production of hypochloraemia (Selye & Dosne, 1940). All these changes have been observed in relatively short term experiments in which treatment was given only for 20 days or even shorter periods. In the present experimental series, which was mainly designed for the study of adaptation to desoxycorticosterone acetate treatment, we examined these responses after two months of daily injections.

EXPERIMENTAL

In the first experiment, twelve male and twelve female young albino rats were used. Their average body weight was 51 g. (range 45–60 g.). They were divided into two groups, each consisting of six males and six females. The first group received daily subcutaneous injections of 2 mg. of desoxycorticosterone acetate in 0·1 ml. of peanut oil during the first 20 days, and 4 mg. in 0·2 ml. daily during the rest of the experiment. The six males and six females of the second group received the same amount of peanut oil and served as controls. After two months of daily treatment, all animals were killed by bleeding from the jugular vein and carotid artery. The blood was used for the determination of its chloride content, the chlorides being determined separately in a sample of plasma and in a sample of whole blood with the Van Slyke method. All organs which appeared of interest in connexion with the problem under investigation were carefully dissected and weighed on a torsion balance after complete fixation in Heidenhain's Susa mixture. Table 1 gives the average organ weights in mg. of each group of six animals and the average whole blood and plasma chlorides expressed in mg. of NaCl/100 ml. The significance of the apparent difference between treated and control animals was evaluated by 'Student's' method for small samples and was expressed in terms of probability estimated by graphic interpolation in Fisher's table of t (Fisher, 1936). It is generally

agreed that differences may be regarded as significant if P is smaller than 0·05. Values showing a statistically significant deviation from the normal are printed in italics in Table 1.

It appears evident that, although the final body weight of the treated animals is approximately the same as that of the controls, many of their organs show significant deviations from the normal weight. Thus the thymus, spleen and kidneys are enlarged in the desoxycorticosterone acetate treated animals of both sexes. This enlargement proved to be statistically significant in all cases with the exception of the thymus of the treated males which, although above normal weight, did not show a statistically significant increase. On the other hand, the adrenal weights as well as the whole blood and plasma chloride levels were significantly decreased by the treatment. This experiment appears to indicate that, although most of the actions of the cortical hormone were maintained during this prolonged period of treatment, its ability to cause thymus and spleen atrophy was not only lost but actually reversed since the

Table 1. *Effect of chronic desoxycorticosterone acetate treatment on organ weights and blood chlorides*

	Desoxycorticosterone acetate		Controls	
	Males	Females	Males	Females
Thymus	395	*435*	351	287
Spleen	*1349*	*944*	960	849
Kidneys	*1896*	*1519*	1501	1156
Adrenals	*18*	*23*	30	42
Whole blood chlorides	*326*	*334*	398	399
Plasma chlorides	*398*	*444*	526	517
Body weight	185	144	199	166

thymus of the treated animals proved to be heavier than in normals. Such a reversal of a hormone effect after continued treatment with a hormone preparation has first been observed in studies concerning the action of parathyroid extracts on bone formation. It was shown that while acute over-dosage with such preparations causes bone absorption and a condition resembling osteitis fibrosa, chronic administration of the same doses of the same extract has exactly the opposite effect inasmuch as it stimulates new bone formation and results in changes resembling the so-called marble bone disease (Selye, 1932). Other instances of inversion of the response to hormone preparations have been discussed elsewhere (Collip *et al.* 1940). However, in the present case we are dealing with a rather special instance of an inverse hormone action because only the thymus and the spleen reacted in this manner while the other responses to the hormone were the same as those following short periods of treatment. Because of the unusual nature of this dissociated adaptation to a hormone effect in which only certain organs become refractory to the usual action of an endocrine principle, a second similar experiment was performed in which a group of 24 very young albino rats having an average weight of 21 g. (range 17–24 g.)

was divided into two groups, each consisting of six males and six females. The animals in the first group received 1 mg. of desoxycorticosterone acetate per day during the first week, this amount being increased by 1 mg. each week until the dose of 5 mg. per day was reached; then injections were continued daily with the same dose until the end of the experiment. The compound was injected subcutaneously, the daily dose being dissolved in 1/20th to 2/10ths of peanut oil depending on the amount administered. The controls received the same amount of peanut oil as was given as a solvent for the desoxycorticosterone acetate to the experimental animals. Table 2 summarizes the results of these experiments, all designations being the same as in Table 1.

The data presented in Table 2 clearly indicate that here again the thymus and spleen reacted inversely inasmuch as they showed a significant increase in weight instead of the usual atrophy. On the other hand, the kidneys responded with an enlargement and the adrenals with a decrease in weight just as in short-term experiments. The whole blood chlorides likewise responded with the usual decrease. In this experiment, plasma chlorides have not been separately

Table 2. *Effect of chronic desoxycorticosterone acetate treatment on organ weights and blood chlorides*

	Desoxycorticosterone acetate		Controls	
	Males	Females	Males	Females
Thymus	*305*	*301*	242	238
Spleen	*921*	*1121*	452	461
Kidneys	*1859*	1353	1325	1184
Adrenals	*13*	*19*	30	49
Whole blood chlorides	*310*	*325*	412	400
Body weight	187	139	191	155

determined since the results of the previous series clearly indicated that the hypochloraemia is demonstrable both in plasma and in whole blood but is more obvious in the latter. All these deviations from the normal proved to be statistically significant with the exception of the renal enlargement in the treated females. The body weight of the treated animals did not prove to be significantly less than that of the controls.

DISCUSSION

The results of the above two experiments show that continued treatment with a hormonally active principle such as desoxycorticosterone acetate may lead to a change in the responsiveness of certain organs (thymus, spleen) without influencing the sensitivity of others (adrenals, kidneys). Whether the fact that the thymus and spleen actually increase in size under the influence of chronic desoxycorticosterone acetate treatment should be interpreted as definite proof of a true inversion of the hormone effect remains to be seen. The adrenals were atrophic in those animals which showed enlargement of the spleen and thymus, and histological sections showed this to be due almost

entirely to atrophy of the cortical cells. It appears possible, therefore, that in the course of chronic treatment with desoxycorticosterone acetate, the thymus and spleen merely become resistant to the atrophy-producing action of this compound, and that the subsequent enlargement of these organs is due to a functional inactivation of the adrenal cortical cells and not to a direct stimulation of the thymus and spleen by desoxycorticosterone acetate. It is felt, therefore, that it is better to speak simply of a 'dissociated adaptation' of the lymphatic organs rather than of an inverse response of the same.

That a dissociated adaptation to a certain action of the hormone may be produced without inducing resistance to all of its actions is also shown by other observations. Thus it has recently been discovered (Selye, 1941 *c*, *d*) that progesterone, desoxycorticosterone acetate, and various androgens cause general anaesthesia if administered intraperitoneally or intravenously to experimental animals. No such effect is observed, however, following subcutaneous administration. It has been assumed that this effect is seen following intraperitoneal or intravenous injection because a rapid flooding of the body with steroid hormones is necessary to obtain the anaesthetic effect. Yet it was found that animals adapted to the anaesthetic action following repeated intraperitoneal administration of a steroid hormone are still sensitive to its other effects and, vice versa, anaesthesia may still be obtained by intraperitoneal administration in animals which had been rendered resistant to those actions of the hormone which are elicited by subcutaneous administration. It is felt in the light of these observations that, following chronic treatment with certain steroid hormones, some organs may become resistant to their action although other organs remain sensitive; that is to say, that the organism may acquire what might be termed a 'dissociated adaptation' to steroid hormones.

SUMMARY AND CONCLUSIONS

Experiments with albino rats show that following prolonged treatment with daily injections of desoxycorticosterone acetate, the animals lose their responsiveness to certain actions of this compound while they remain sensitive to its other effects. Short term treatment with this adrenal cortical principle causes involution of the thymus, spleen, and adrenal cortex, accompanied by enlargement of the kidneys and a decrease in blood chlorides, while long term treatment with the same substance results in enlargement of the thymus and spleen even though the other changes are the same as those observed in short-term experiments.

It remains doubtful whether the enlargement of the thymus and spleen which occurs in these experiments can be considered a true inversion of the hormone action. It is possible that these lymphatic organs merely become resistant to the atrophy-producing effect of desoxycorticosterone acetate, and that their weight increases above that of untreated controls merely because the desoxycorticosterone acetate causes adrenal cortical atrophy and the lymphatic organs enlarge as a consequence of the functional inactivation of the

adrenal cortical cells. However, in any case, the experiments here described show that a 'dissociated adaptation' to a hormone action may be acquired by a certain organ without there being any change in the sensitivity of the body to other effects of this principle.

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REFERENCES

CARRIERE, G., MOREL, J. & GINESTE, P. J. (1937). *C.R. Soc. Biol., Paris*, **126**, 46.
COLLIP, J. B., SELYE, H. & THOMSON, D. L. (1940). *Biol. Rev.* **15**, 1.
FISHER, R. A. (1936). *Statistical Methods for Research Workers*, 6th ed. p. 128. Edinburgh.
KENDALL, E. C. (1940). *Proc. Amer. Soc. Biol. Chem.* New Orleans.
LOW, F. N. (1938). *Endocrinology*, **22**, 443.
SELYE, H. (1932). *Endocrinology*, **16**, 547.
—— (1936). *Brit. J. exp. Path.* **17**, 234.
—— (1940*a*). *Amer. J. Physiol.* **130**, 358.
—— (1940*b*). *Canad. med. Ass. J.* **42**, 113.
—— (1940*c*). *J. Amer. med. Ass.* **115**, 2246.
—— (1941*a*). *J. Pharm. exp. Ther.* (in the Press).
—— (1941*b*). *J. Urol.* (in the Press).
—— (1941*c*). *Proc. Soc. exp. Biol., N.Y.*, **46**, 116.
—— (1941*d*). *J. Pharm. exp. Ther.* **71**, 236.
SELYE, H., COLLIP, J. B. & THOMSON, D. L. (1935). *Proc. Soc. exp. Biol., N.Y.*, **32**, 1377.
SELYE, H. & DOSNE, C. (1940). *Proc. Soc. exp. Biol., N.Y.*, **44**, 165.
WELLS, B. B. & KENDALL, E. C. (1940*a*). *Proc. Staff Meet. Mayo Clin.* **15**, 133.
—— —— (1940*b*). *Proc. Staff Meet. Mayo Clin.* **15**, 324.

OBSERVATIONS ON THE CARDIO-VASCULAR SYSTEM AND LUNGS OF AN AFRICAN ELEPHANT FOETUS

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From the Royal Veterinary College, London, and the Nuffield Institute for Medical Research, Oxford

In collaboration with Dr A. E. Barclay, we are making an extensive comparative study of the foetal cardio-vascular system of eutherian mammals. The present paper comprises part of that study. It is published separately because elephant foetuses are extremely rare, and it is therefore of importance that any fresh account should be as detailed, and as well illustrated, as possible. So far as we are aware, the only previous descriptions of the foetal circulatory system of Proboscidea have been those of Eales (African elephant) and of Osman Hill (Asiatic elephant). These observers, however, were concerned with the descriptive anatomy of the parts, while we have been primarily interested in them from their functional aspect. We disagree with certain statements and pictured details in Eales's account, and from our point of view it contains lacunae; nevertheless, we should like to acknowledge the help that we have received from this pioneer work. Hill's account concerns a different species of elephant, and it covers relatively little common ground; it provides, therefore, much less opportunity for comment.

The reasons for our comparative study are as follows. In co-operation with Dr A. E. Barclay, Sir Joseph Barcroft and Dr D. H. Barron, two of us produced the first direct records of the circulation in the intact foetus, with the placental circulation continuing (Barclay *et al.* 1939, 1941). Later, we made an extensive search through the literature (Franklin, 1941, Part I), and in the light of this and of our own findings we planned not only a third season of experimental work, but also a correlation of the anatomy of our subject (the nearly full-term lamb, delivered by Caesarean section) with its physiology (Franklin *et al.* 1940, and unpublished). Our experimental technique (Barclay *et al.* 1940), however, has two drawbacks that preclude its use over a wide range of subjects. The first disadvantage is its expense, the second that we cannot with any convenience work upon foetuses either very much larger or very much smaller than the full-term lamb. Despite these obstacles, we should probably have gone on to another species of mammal to corroborate the findings in our forty or so lambs, had not the war made such an objective impossible of attainment. Our plan, therefore, has been to ascertain the essential anatomical features that are associated with the physiological picture in the lamb, and then to see if these features are present in the foetuses of other mammals. So far we have dissected about a hundred specimens belonging to

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nineteen different genera, and the essential structures have been present in every case.

The subject that provides the material for this present paper is a well-preserved male African elephant foetus. So was that dissected by Eales. Our specimen is very slightly smaller than hers, the age of which she gave (Eales, 1926*b*) as about 10 months, i.e. somewhat less than half way to full term. Pl. 1, fig. 1 shows the external appearance of our specimen. The total length of umbilical cord attached to the specimen was about 16 cm.; the placenta was not available.

We may now pass to the detailed description of the parts with which we are concerned:[1]

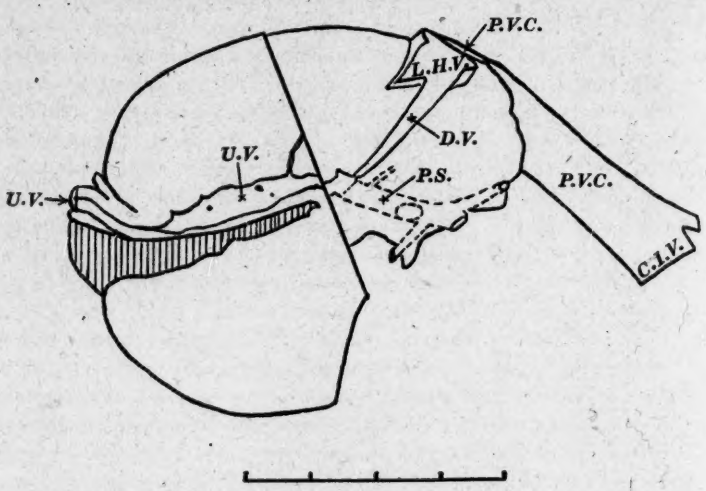
(1) *The umbilical vein.* This is single and valveless. In its extra-abdominal course it is very thick-walled, and its lumen decreases towards the umbilicus. About 2 cm. before reaching the umbilicus (Pl. 2, fig. 4), its wall is of irregular width (0·35 cm. at the widest part), and the longest and shortest diameters of its lumen are 0·3 and 0·1 cm. respectively. After passing through the abdominal wall of the foetus, the vein bends sharply at almost a right angle and passes anteriorly, without any appreciable lateral deviation, towards the liver. The thickness of the wall of the vessel decreases to 0·2 cm., and the lumen disappears, anterior to the intra-abdominal bend. From the bend to its entry into its hepatic fossa, the length of the vein is about 2·5 cm. The rest of its course will be dealt with in the next section.

(2) *The liver.* Before removing the liver, we made a cut on its ventral surface in the long axis of the abdomen, and a piece of cotton was laid in the cut before we took our photographs (see Pl. 2, fig. 4). Our description of the organ does not agree with that of Eales (1929), nor do our photographs agree with her drawing; on the other hand, our division of the liver into lobes is in line with the venous distribution. In our foetus the greater part of the liver lies to the left of the abdominal mid-line and the viscus extends farther dorsally on the left side; this latter fact may be correlated with the greater posterior extension of the right lung (Pl. 2, fig. 2). A very deep fissure on the posterior aspect of the liver passes ventrally, and then to the left and anteriorly, to its end on the anterior surface (Pl. 2, fig. 4). This fissure divides the liver into right and left lobes in such a way that the degree of separation of these lobes exceeds their degree of union. The right lobe appears somewhat greater than the left one. Two much shallower fissures, mainly on the posterior surface, partially subdivide the right lobe into dorso-lateral and ventral portions, with a small,

[1] (1) In our description we visualize the foetus in the pronograde position, and use the terms anterior and posterior, dorsal and sternal or ventral, and right and left, in relation to this position.

(2) In more than one instance a vessel in our specimen is markedly contracted over a part or the whole of its length; the lumen is correspondingly reduced, sometimes to vanishing point. Such reductions in calibre must have occurred *post mortem*, either naturally, or through the agency of the fixative used, or through a combination of both these processes. The contractions are of value in that they indicate the relative musculo-elastic reactivity of the vessels, or parts of vessels, and so make it possible to envisage their physiology.

tongue-shaped piece between them. The ventral portion extends to the left of the abdominal mid-line, and the fossa into which the umbilical vein enters lies more or less in the middle of it from right to left. The line of the suspensory ligament (Pl. 2, fig. 4) is parallel to the long axis of the abdomen. There is no gall-bladder. The portal vein also enters the right lobe of the liver. In the anterior view of the organ, at the immediate post-diaphragmatic level, can be seen the left hepatic vein about to unite with the posterior vena cava, which has already united with first the right, and then the middle, hepatic vein. The total length of the abdominal portion of the posterior vena cava is small (about 4 cm.), for in the elephant the common iliac veins do not unite until they have



Text-fig. 1. Left lateral view of a central slice of the liver. To the right of the oblique line the liver has been cut away to a depth of 0·25 cm. Hatched area, external liver surface. *U.V.* umbilical vein, contracted outside liver, dilated inside. *D.V.* ductus venosus. *L.H.V.* left hepatic vein. *P.V.C.* posterior vena cava. *C.I.V.* common iliac vein. *P.S.* 'portal sinus'. This sinus, the three vessels forming the portal vein, and the two efferents from the sinus, are indicated by interrupted lines. Centimetre scale.

passed exceptionally far forward in the abdomen (see Text-fig. 1, and Eales, 1929, fig. 6). The vena cava has no appreciable intra-hepatic course; hence there are no small hepatic veins entering it posteriorly to the three large veins.

We exposed the essential internal features of the liver by two main cuts, two subsidiary ones, and a little further dissection. The two main cuts were made ventro-dorsally, parallel to the long axis of the abdomen and 1·0 cm. apart; the left-hand cut divided the umbilical vein along its length at its entry into the liver (Text-fig. 1). To expose the ductus venosus, a slice 0·25 cm. in thickness was removed, by two straight cuts, from the left-hand dorsal portion of the central slice of liver already secured by the earlier cuts. Finally, three

afferent veins forming the portal vein, together with the so-called 'portal sinus', were slit up along their length. The hepatic venous distribution was ascertained by the passing of probes.

The umbilical vein preserves the thick walls characteristic of its extra-hepatic course until it has properly entered the liver. It then broadens out into a wide, thin-walled vessel and over a course of some 2·5 cm. gives off branches right and left to the ventral portion of the right lobe. Thereafter it divides into two terminal branches of larger size. Of these one (not shown in Text-fig. 1) passes slightly to the left and anteriorly to distribute its branches to the left lobe of the liver, the other turns slightly right and posteriorly to unite with the 'portal sinus'. Just before this union the ductus venosus opening is visible.

The ductus has at its origin a slight ridge projecting into the lumen. We have seen a similar ridge in the ductus venosus of the foetal lamb and there is a suggestion in our experimental records that closure of the ductus may be effected at the level of this ridge, with side-to-side flattening of the rest of the ductus. We have not published our findings in the lamb, for we wish not to advance a definite hypothesis until we have a series instead of a few instances, and we also await the results of a histological study at present being made by Dr D. H. Barron. It is, however, of interest that the ductus venosus of our elephant foetus has a corresponding ridge and is flattened from side to side throughout its length. Apart from these resemblances, the ductus in the elephant is not similar to that in the lamb. It runs an almost straight course from the umbilical vein to the right side of the left hepatic vein, and does not deviate to the left or to the right. Viewed from the side, it shows a progressive increase in width (0·1–0·35 cm.) as it passes from its origin to its termination.

The 'portal sinus' is a short channel that gives off a large efferent vessel to the right near its portal end and a much smaller efferent vessel dorsally, and slightly to the right and anteriorly, near its umbilical end. These two off-shoots supply branches to the dorso-lateral portion of the right lobe of the liver, a distribution comparable, mutatis mutandis, with that which we found in the foetal lamb.

The hepatic veins are three in number. Of these the right is formed by branches draining the dorso-lateral portion of the right lobe of the liver, the middle by branches having the same function in respect of the right half of the ventral portion of the same lobe. The left half of this portion, and the entire left lobe, are the territory of the left hepatic vein, which also, as we have said above, receives the ductus venosus. In correspondence with the relative sizes of their drainage areas, the left hepatic vein is the largest of the three and the middle one the smallest.

Immediately anterior to the diaphragm the posterior vena cava is a single vessel, i.e. the union of the left hepatic vein with it has occurred about the level of the diaphragm.

(3) *The intrathoracic viscera.* The details of the heart and lungs are not immediately visible when the thorax is opened, on account of the thickness

and opacity of the pericardium and parietal pleura. The latter is attached to the surrounding structures by a fairly copious areolar connective tissue that allows easy separation of the pleura with the finger-tips.

On removing the parietal pleura, we found that it was not attached to the visceral pleura in any way; in their adult specimen Miall & Greenwood (1878, p. 44) found the two layers closely attached by 'matted elastic tissue'. Before proceeding further, we took photographs of the heart and lungs from different angles; four of these are reproduced in Pl. 1, fig. 2, and Pl. 2, figs. 1, 2 and 3.

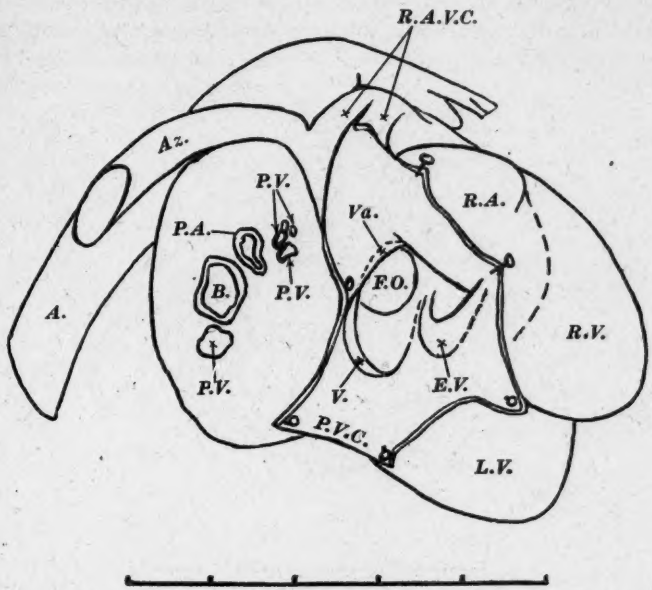
(*a*) *The lungs*. These are nowhere adherent to one another as they are in some animals, e.g. the horse (Sisson, 1940, p. 541, and Figs. 474, 475). The right lung is larger than the left. In other mammals we have found relations between the pulmonary venous drainage and the lobes of the lungs. In the foetal elephant it is not very easy to identify particular lobes because the lungs are composed of numerous small, medium, and large lobules, and the main visceral pleura bridges the superficial fissures between these instead of dipping down into them, though there appears to be some more delicate investment of the lobules themselves. We think, however, that we can identify more or less accurately, on both sides, apical, cardiac, and diaphragmatic lobes (veterinary terminology, as used by Sisson, 1940), and belonging to the right lung, in addition, an intermediate (or azygos, or mediastinal) lobe. Our tentative identification of the lobes is given in Pl. 2, figs. 1–3.

(*b*) *The heart and great vessels* (Text-figs. 2–4, and Pls. 1 and 2). The most striking feature in the external appearance of the heart is the wide separation of the apices of the two ventricles and the consequently marked interventricular notch. This feature is more pronounced in our specimen than in those of Eales and of Hill, and in ours, also, there is a marked difference in colour between the ventricles. The notch lies just to the right of the mid-line of the thorax.

The elephant is one of those mammals which retain both right and left anterior venae cavae. The right one (Text-figs. 2, 4) receives the azygos venous drainage (see Hill for differences between African and Asiatic types). The left one passes posteriorly along the dorsal border of the left atrium and then turns to the right to reach the right atrium (Text-figs. 3, 4). It receives near this bend three separate coronary veins. These latter have occasional unicuspid parietal valves, but no ostial valves.

The coronary sulci contain, in addition to the vessels, a large amount of very vascular foetal connective tissue with ganglionic masses and nerve cells with fibres; there is no fat. A tongue-like projection extends over the antero-sternal aspect of the origin of the aorta (Pl. 1, fig. 2). Eales (1929, p. 207) wrote of her specimen that 'the coronary grooves are pronounced, and contain, in addition to the coronary arteries and veins, a small amount of fat'. Retzer (1912, p. 76) said of his adult specimen that 'a marked oedema round the coronary sulcus obliterates it in places'.

The majority of the internal features of the posterior vena cava, right atrium, and right anterior vena cava were exposed by a single cut, as shown in Text-fig. 2. The essential features of the left side of the heart, namely, the valve of the foramen ovale and its relation to the pulmonary vein entries, were exposed by removal of much of the left wall of the atrium and ventricle, but



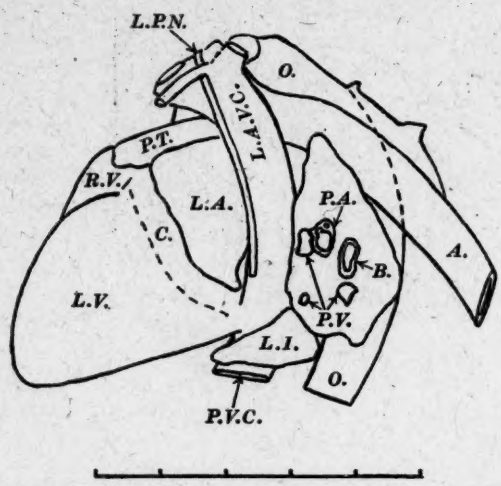
Text-fig. 2. Drawing, made from a photograph of the right side of the heart and great vessels and the cut right lung. The posterior and right anterior venae cavae are opened up and retracted. *P.V.C.* posterior vena cava. *Az.* azygos vein. *R.A.V.C.* right anterior vena cava. *E.V.* Eustachian valve. *V.* other cusp of bicuspid valve of *P.V.C.* *F.O.* Foramen ovale. *Va.* 'valve' of *R.A.V.C.* *R.A.* right atrium. *R.V.*, *L.V.* right and left ventricles. *A.* aorta. *B.* right bronchus. *P.A.* right pulmonary artery. *P.V.* pulmonary veins; the lowest from diaphragmatic lobe, the next lowest from cardiac lobe, and the top veins from apical lobe. Centimetre scale.

are not easily shown except by stereophotographs; so we present no illustration with this account.

The combined picture is similar in essential features to what we have found in all the eutherian foetal hearts that we have examined, though it is slightly complicated by the presence of the left anterior vena cava and of the extra valvular structures on the right side.

The thoracic portion of the posterior vena cava is relatively longer than in the human foetus but shorter than in those of most mammals. *The posterior*

caval channel,[1] by which we mean not only the vena cava proper but also its physiological continuation within the heart, divides on a marked ridge into *right* and *left terminal divisions*. This ridge, which we name *crista dividens*, has been a very prominent feature in all the mammalian foetuses that we have examined; its lie varies somewhat in different species, and it is distinct from muscle bundles such as those of the limbus fossae ovalis, tuberculum intervenosum, etc., though it may unite in part of its course with one or more of these structures. Physiologically, this crest is of fundamental importance, for the posterior caval stream divides on it (Barclay *et al.* 1939, and unpublished);

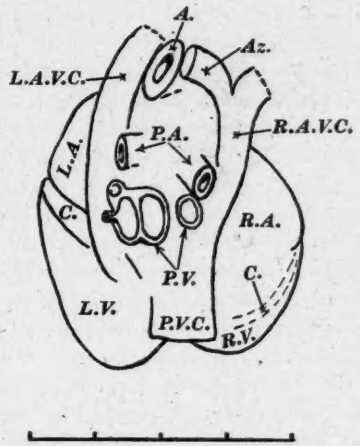


Text-fig. 3. Left lateral view of the heart and great vessels and the cut left lung. *P.V.C.* posterior vena cava. *L.A.V.C.* left anterior vena cava. *L.P.N.* left phrenic nerve. *L.I.* lobus intermedius of right lung. *O.* oesophagus. *B.* left bronchus. *C.* coronary sulcus. *P.A.* left pulmonary artery. *P.V.* pulmonary veins, upper one from apical lobe, left lower one from cardiac lobe, right lower one from diaphragmatic lobe. *L.A.* left atrium. *L.V.* left ventricle. *R.V.* right ventricle. *P.T.* pulmonary trunk. *A.* aorta. Centimetre scale.

it has, however, received relatively little attention from most previous observers, with the exception of Wolff, Kilian, and Ziegenspeck, and perhaps one or two others. Unfortunately for progress, their correct appreciation of certain anatomical features had no experimental support, and for this and for other reasons they failed to influence, to any significant extent, either their contemporaries or their successors. The left terminal division of the posterior caval channel is bounded by a *fixed portion* and by a *free* or *apposable portion* (the so-called valve of the foramen ovale). The former in our elephant foetus is

[1] The terminology used by us in this and subsequent paragraphs is based upon a correlation of the anatomy and physiology in the foetal lamb. We hope to give a longer account of it at some future date.

part of the interatrial septum. The apposable portion is so called because after birth it is apposed and, according to its size, either blocks the left terminal division completely or else reduces its calibre. In our specimen this portion is of moderate length and its anterior half is fenestrated. It is more complex than the corresponding structure in the average human subject but far less so than those in some mammals (e.g. the ox and the horse). The short right terminal division of the posterior caval channel is separated, on the dorsal aspect, from the termination of the *right anterior caval channel* by a fairly pronounced tuberculum intervenosum or, as Sisson styles it, crista intervenosa; we prefer the term *crista interveniens*.



Text-fig. 4. Dorsal view of the heart and great vessels. *P.V.C.*, *R.A.V.C.*, *L.A.V.C.* posterior and right and left anterior venae cavae. *Az.* azygos vein. *R.A.*, *L.A.* right and left atria. *R.V.*, *L.V.* right and left ventricles. *A.* aorta. *P.A.* right and left pulmonary arteries. *C.* coronary sulci. *P.V.* five pulmonary vein entries. The two largest are from right and left diaphragmatic lobes; upper small left from left apical lobe, lower small left from left cardiac lobe; medium-sized entry on right from right apical and cardiac lobes. Centimetre scale.

In our specimen there is what appears to be a bicuspid valve (see Text-fig. 2) of the posterior caval channel. One cusp lies to the left and somewhat dorsally, the other cusp (the Eustachian valve) to the right and somewhat ventrally. The left one resembles a poorly developed cusp of a type often found in the course of a vein. It begins where the 'valve' of the right anterior vena cava ends (see below), and curves in succession to the left and posteriorly, then sternally and anteriorly, and finally anteriorly. In the first half of its course it is shallow enough from its attached to its free border; in the latter part it becomes merely a line along the surface of the atrium. This line runs along one side of a projecting bundle of muscle, identified as part of the fleshy structure surrounding the entry of the left anterior vena cava into the right atrium. The other side of

the projecting bundle of muscle carries the attached border of part of the Eustachian valve. In other words, the muscle bundle acts as one of the two cornua of the bicuspid valve (if we are right in so styling it) of the posterior caval channel. The Eustachian valve, or right cusp of the bicuspid valve, is well developed and separates the posterior caval channel from the *left anterior caval channel*. In our long series of mammalian foetuses, well-developed Eustachian valves have been very rare, and a bicuspid valve, of which the Eustachian valve forms one cusp, rarer still. It is for these reasons, and also because the valves in the adult elephant have been somewhat obscurely described, that we have given the above detailed account of the appearances in our foetal specimen.

The 'valve' of the right anterior vena cava may be regarded functionally as an extension of the crista interveniens. Eales found it in her foetus, but it was not present in that of Hill. In the adult it was described by Vulpian and Philipeaux, Watson, and Miall and Greenwood; it was pictured by Watson. In our specimen it begins ventrally on the wall of the right atrium about a third of the way across from the right side to the septum (the beginning of the 'valve' is not visible in Text-fig. 2). Thence it passes to the left and dorsally, and at the same time increases in width from its attached to its free border, while the attached border itself joins the thick rounded crista terminalis. Where the crista terminalis merges into the crista interveniens, the attached border transfers to the latter and the distance between the attached and free borders slowly diminishes until, at the termination of the valve and of the crista interveniens, they meet. In correspondence with its attachment, the valve, in the last part of its course, turns more sharply dorsally and also posteriorly, and finishes on the dorsal aspect of the right anterior vena cava, and to the right of the dorso-ventral mid-line of the vessel.

The crista dividens is quite prominent in its middle portion. Ventrally it fuses with the more medial cornu (see above) of the bicuspid valve of the posterior caval channel, and dorsally with the crista interveniens, posterior to the union of the crista terminalis with this latter. Between the crista dividens and the 'valve' of the right anterior vena cava is a fossa or pocket. Hill (1938*b*, p. 48) appears to be referring to our crista dividens when he describes the foramen ovale as 'guarded by an arched muscular margin, especially above'.

The pulmonary venous drainage is as follows (Text-figs. 2–4). The veins from the right apical lobe unite near the hilus, and the single vein so formed soon after unites with the vein from the right cardiac lobe and enters the left atrium on its dorsal aspect by a separate entry of fairly large size. The vein from the lobus intermedius joins posteriorly, near the heart, the large vein draining the diaphragmatic lobe, or main bulk, of the right lung. This large vein enters the left atrium to the left of the vein already described. The vein draining the left apical lobe, and also one or two lobules of the cardiac lobe, enters the left atrium by the smallest but one separate entry. The vein draining the bulk of the left cardiac lobe has the smallest separate entry, if indeed it

has not actually joined with the main vein of the left lung before entering the atrium. The large vein draining the diaphragmatic lobe, or main bulk, of the left lung enters the atrium posteriorly and to the right of the two small veins just described, and is separated by only a thin septum at this point from the corresponding large vein from the right lung. It is obvious, if our account of the entries is compared with those of Eales and Hill in respect of foetal hearts, and of others in respect of adult hearts, that their number is variable. That is a matter of little concern. What is of much greater importance is that the total pulmonary inflow must impinge upon the valve of the foramen ovale, or apposable portion of the left terminal division of the posterior caval channel. In many foetuses the inflow that so impinges is chiefly that from the right apical and cardiac lobes, in a smaller number that from the right cardiac and diaphragmatic lobes. In our elephant foetus, however, all the pulmonary veins enter into a common sinus on the dorsal aspect of the left atrium; thus the whole of the venous inflow must go past the valve of the foramen ovale. The point is of importance in relation to any hypotheses about the functional apposition of the valve after birth.

The pulmonary valve has corpora Arantii on its ventral and right cusps, but there is not one on the left cusp. The aortic valve has a corpus Arantii on each cusp. Eales and Hill made no mention of such structures in their foetuses. In the adult elephant, Vulpian & Philipeaux (1856, pp. 188–9, 191), and after them Miall & Greenwood (1878, p. 38), reported that corpora Arantii were absent from the cusps of the pulmonary valve, and made no mention of any in connexion with the aortic valve. Retzer (1912, p. 83), on the other hand, wrote that 'the aorta and pulmonary arteries do not present any marked peculiarities except the lack of a nodulus valvulae semilunaris (Arantii) in the cusps of pulmonary'.

The pulmonary trunk in our specimen gives off a relatively small branch on its posterior aspect and then continues as the ductus arteriosus. The small branch almost immediately divides into right and left pulmonary arteries. The ductus is normal in length (as compared with those of other genera) and elliptical in cross-section. The long diameter of the ellipse is antero-posterior and measures 0·4 cm.; the short diameter measures 0·1 cm. The lack of any great contraction of the ductus arteriosus after fixation contrasts with the contraction of the extra-hepatic portion of the umbilical vein, of the ductus venosus, and of the greater part of the umbilical arteries. In Eales' specimen (kindly lent to us) the long (antero-posterior) and short diameters of the ductus are about 0·3 and 0·2 cm. respectively. Hill stated that the ductus in his foetus was given off before the pulmonary trunk terminated by dividing into the two pulmonary arteries.

(4) *The umbilical arteries.* The abdominal aorta in our specimen terminates by giving off in succession the external iliac arteries, the umbilical arteries, the caudal artery, and the internal iliac arteries. The paired vessels have external diameters of 0·3, 0·6, and 0·3 cm. respectively, i.e. the total cross-sectional

area of the umbilical vessels is double that of all the iliac vessels. The caudal artery and the internal iliacs are given off from a circular depression between the two umbilical arteries. About 6 cm. from their origin the umbilical arteries show an external narrowing, and from this point they quickly become very thick-walled and their lumina impervious even to a fine probe.

SUMMARY

1. An account is given of the lungs, and of certain features of the cardio-vascular system, of an African elephant foetus. It is the third elephant foetus in which the circulatory system has been studied at all, and the first in which it has been studied from a functional point of view.

2. Previously the anatomical features in the foetal lamb had been correlated with radiographic records of the circulation in that animal. These records are the only direct ones at present available of the blood flow in intact foetuses; they provide, therefore, the sole basis for a functional interpretation of the structure of the foetal cardio-vascular system.

3. The same essential anatomical features were present in our elephant foetus (and in all other mammalian foetuses dissected by us) as in our lamb foetuses.

4. The present morphological nomenclature is not adequate for the description of the foetal heart and great vessels from a functional point of view. Physiologically, we picture a *posterior* (*inferior*) *caval channel* going up into the heart and dividing on a marked crest, the *crista dividens*, into *right and left terminal divisions*, the former directing blood to the right atrium and ventricle, the latter to the left atrium and ventricle. The left terminal division is bounded by a *fixed portion* and an *apposable portion*, the latter being what is commonly called the valve of the foramen ovale, though in many animals, particularly the horse and the ox, it has no resemblance at all to a valve in the generally accepted sense of that term. After birth the apposable portion is apposed and the left terminal division of the posterior (inferior) caval channel either ceases to exist or is considerably reduced in calibre at its termination. The right terminal division of the posterior (inferior) caval channel is separated dorsally from the *anterior* (*superior*) *caval channel* (or right anterior caval channel, where two anterior venae cavae are present) by the tuberculum intervenosum or, as we prefer to style it, the *crista interveniens*. We suggest the above terminology in the light of our direct records of the foetal circulation, of our anatomical studies of foetuses from nineteen eutherian genera, and of a comprehensive search through the literature from Galen's time to the present century.

5. We have included certain other features in the description of our elephant foetus in view of statements made by previous writers about foetal or adult specimens dissected by them.

Fig. 1

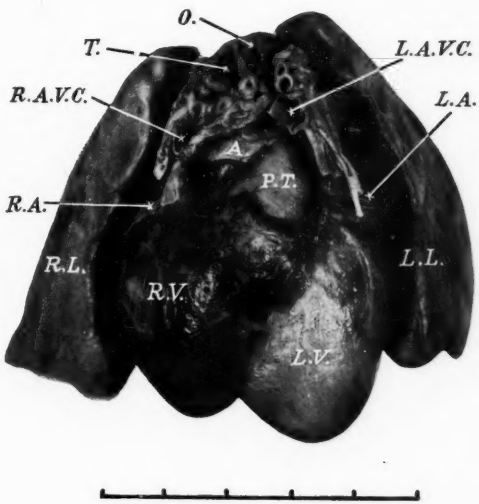


Fig. 2

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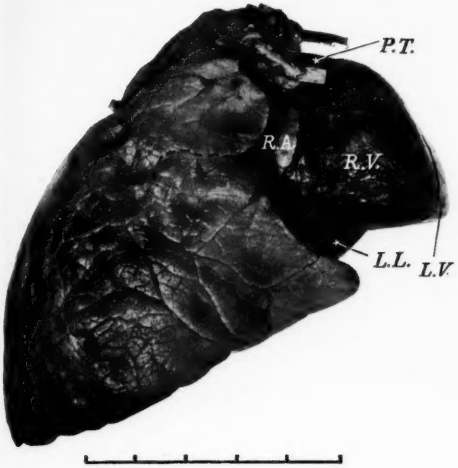

Fig. 1

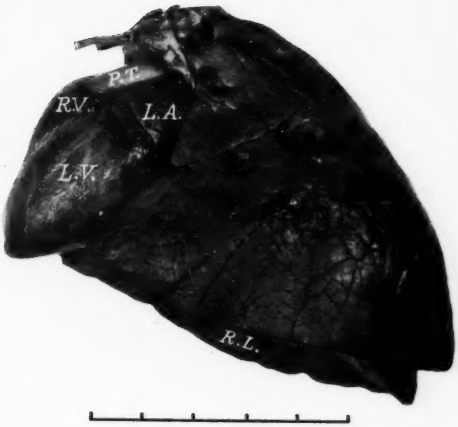

Fig. 2

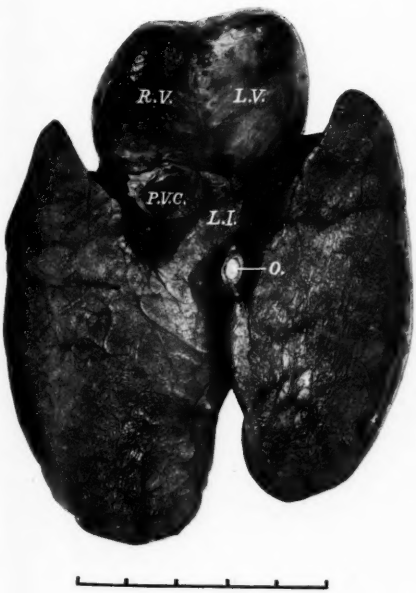

Fig. 3

Fig. 4

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REFERENCES

Barclay, A. E., Barcroft, J., Barron, D. H. & Franklin, K. J. (1939). *Brit. J. Radiol.* **12**, 505–17.

Barclay, A. E., Barcroft, J., Barron, D. H., Franklin, K. J. & Prichard, M. M. L. (1941). *Amer. J. Anat.* (in the Press).

Barclay, A. E., Franklin, K. J. & Prichard, M. M. L. (1940). *Brit. J. Radiol.* **13**, 227–34.

Eales, N. B. (1925). *Proc. Zool. Soc. Lond.* pp. 445–56.

—— (1926*a*). *Trans. Roy. Soc. Edinb.* **54**, 491–551.

—— (1926*b*). *Anat. Anz.* **61**, 361–4.

—— (1928). *Trans. Roy. Soc. Edinb.* **55**, 609–42.

—— (1929). *Trans. Roy. Soc. Edinb.* **56**, 203–46.

—— (1931). *Proc. Zool. Soc. Lond.* pp. 115–25.

Franklin, K. J. (1941). *Ann. Sci.* **5**, 57–89.

Franklin, K. J., Barclay, A. E. & Prichard, M. M. L. (1940). *J. Anat., Lond.*, **75**, 75–87.

Hill, W. C. O. (1938*a*). *Ceylon J. Sci.* **21**, 31–43.

—— (1938*b*). *Ceylon J. Sci.* **21**, 45–61.

Kilian, H. F. (1826). *Ueber den Kreislauf des Blutes im Kinde, welches noch nicht geathmet hat.* Karlsruhe: Verlag der Chr. Fr. Müller'schen Hofbuchhandlung.

Miall, L. C. & Greenwood, F. (1878). *J. Anat., Lond.*, **13**, 17–50.

Retzer, R. (1912). *Anat. Rec.* **6**, 75–90.

Sisson, S. (1940). *The anatomy of the domestic animals*, 3rd ed., revised by J. D. Grossman. Philadelphia and London: W. B. Saunders Co.

Vulpian & Philipeaux (1856). *Ann. Sci. nat., Paris*, 4*s.*, Zool., **5**, 183–204.

Watson, M. (1871). *J. Anat., Lond.*, **6**, 82–94.

Wolff, C. F. (1776). *Novi Commentarii Acad. Sci. Imp. Petropolitanae*, Tom. xx pro Anno mdcclxxv, 357–430.

Ziegenspeck, R. (1884). Beilage iii, pp. 596–607 in Preyer, W. (1885). *Specielle Physiologie des Embryo.* Leipzig: Th. Grieben's Verlag (L. Fernau).

—— (1905). *Samml. klin. Vorträge*, Ser. xiv, 405.

EXPLANATION OF PLATES 1 AND 2

Plate 1

Fig. 1. Right lateral view of male African elephant foetus. Centimetre scale.

Fig. 2. Antero-sternal view of heart and lungs. *R.L.* right lung. *L.L.* left lung. *R.A.V.C.* and *L.A.V.C.* right and left anterior venae cavae. *R.A.* right atrium. *R.V.* right ventricle. *L.A.* left atrium. *L.V.* left ventricle. *P.T.* pulmonary trunk. *A.* aorta. *T.* trachea. *O.* oesophagus. Centimetre scale.

Plate 2

Fig. 1. Right lateral view of heart and lungs. On the right lung interrupted lines have been placed between the apical and cardiac, and cardiac and diaphragmatic lobes respectively. *R.A.* right atrium. *R.V.* right ventricle. *L.V.* left ventricle. *P.T.* pulmonary trunk. *L.L.* left lung. Centimetre scale.

Fig. 2. Left lateral view of heart and lungs. On the left lung, interrupted lines have been placed between the apical and cardiac, and cardiac and diaphragmatic lobes respectively. *L.A.* left atrium. *L.V.* left ventricle. *R.V.* right ventricle. *P.T.* pulmonary trunk. *R.L.* right lung. Centimetre scale.

Fig. 3. Posterior view of heart and lungs. Interrupted lines have been placed on each lung between the cardiac and diaphragmatic lobes. *L.I.* lobus intermedius (or azygos or mediastinal lobe) of right lung. *P.V.C.* posterior vena cava. *O.* oesophagus. *R.V.* right ventricle. *L.V.* left ventricle. Centimetre scale.

Fig. 4. Ventral view of the liver, umbilical vein, and part of umbilical cord. The long axis of the trunk is indicated by a piece of cotton. Interrupted lines mark fissures dividing the liver into lobes and subdividing the right lobe. *P.V.C.* posterior vena cava. *L.H.V.* left hepatic vein. *U.V.* umbilical vein. *U.A.* umbilical artery. *U.* urachus. Centimetre scale.

THE DEVELOPMENT AND SIGNIFICANCE OF THE CELL COLUMNS IN THE VENTRAL HORN OF THE CERVICAL AND UPPER THORACIC SPINAL CORD OF THE RABBIT

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Although a great deal of attention has been paid to the arrangement of the cell groups or columns in the spinal cord (Steida, 1870; Obersteiner, 1890; Schieffer-decker, 1874; Waldeyer, 1889; Kreyssig, 1885; Onuf, 1899; Bruce, 1901; v. Gehuchten & de Neef, 1900; Jacobsohn, 1908; Bok, 1928; Angulo y González, 1927) and to the relation between these columns and the peripheral nerves or muscle masses (Sano, 1897, 1898; Marinesco, 1898*a*; v. Gehuchten & de Buck, 1898; v. Gehuchten & Nelis, 1899; Sass, 1889; Hammond, 1894; de Neef, 1901; Yu-Ch'uan Tsang, 1939), very little is known concerning the development of the cell columns[1] in the spinal cord and the relation between this development and that of the muscles which the processes of these cells grow out to supply. Frazer (1931) mentions that he can see cell groups already present in the ventral horn of an 8 mm. human embryo cut in transverse section, but he does not trace out the development in any great detail.

The purpose of this communication is to describe the development of the cell columns in the cervical enlargement of the rabbit and the relation between this and the development of the peripheral musculature and the nerves in the forelimb.

MATERIAL AND METHODS

Under urethane anaesthesia rabbit embryos of known age were removed by laparotomy from pregnant rabbits. These embryos were studied for signs of spontaneous movements and were stimulated with a weak faradic current to determine the age at which the muscles of the forelimb respond by contraction to direct or reflex stimulation. The embryos collected are enumerated in Table 1.

Small embryos were fixed whole in various fixatives including 95 % alcohol and 2 % acetic acid for staining with toluidin blue, in absolute alcohol and 1 % strong ammonia solution for pyridin-silver staining, and also in 10 % formalin. Larger embryos were injected through the umbilical artery, or the spinal cord was exposed prior to fixation.

[1] Since this paper was completed Angulo y González (1940) has published a study on the white rat in which he has found an arrangement strikingly similar to that in the rabbit.

The spinal cords and dorsal root ganglia were removed intact from the acetic-alcohol fixed embryos, carefully straightened, embedded in paraffin wax, serially sectioned in transverse, coronal and sagittal planes and stained with toluidin blue. Various thicknesses of section were used, the thickest, 20 μ, being used only where it was not required to study the cell structure. In the case of the smallest embryos the whole of the spinal column was removed with the spinal cord to avoid unnecessary damage. The pyridin-silver preparations were cut transversely at 10 μ after impregnation in the silver bath for a period of from 5 to 7 days. This increased impregnation was found to give more satisfactory results in the case of rabbit embryos.

The spinal cords of several adult rabbits were fixed by injection with 10 % formalin, removed together with the spinal roots, and imbedded in paraffin wax. The cervical and upper thoracic regions were sectioned in transverse, coronal and sagittal planes and stained with toluidin blue. Owing to the diffuse nature of the cell columns in the adult rabbit the coronal and sagittal sections were not found so useful as in the earlier stages and were superseded by the transverse sections which were much more easily interpreted.

For the interpretation of the sections in the coronal or sagittal planes of the younger rabbits the glass plate reconstruction method was used.

Table 1

Days from copulation	Crown-rump length of embryos mm.
11	6
12	8
14	11
15	15
16½	19
19	26
21	41
22	56
26	80

NOMENCLATURE

The common nomenclature used to describe the cell columns found in the spinal cord of various animals takes into account the position of these cell columns relative to each other in the ventral or anterior horn of the spinal cord. This nomenclature is only of use in cases where the cell columns are few and simple and where names may be used without drawing homologies with similarly named columns, not necessarily of the same function, in other animals. However, both these difficulties have been encountered in studying the rabbit's spinal cord, and as a result an alternative numerical nomenclature has been used. Similar nomenclatures have already appeared in various publications, and it is stressed here that the numbers used to designate the various cell columns in this study do not correspond with numbers used by other authors. The columns in this study are numbered in order of their appearance in serial transverse sections traced from the first cervical segment

caudally. It should be noted that this nomenclature overcomes the difficulties which arise when a cell column changes its anatomical relationship to the other cell columns in the ventral horn.

CELL COLUMNS IN THE CERVICAL AND UPPER THORACIC REGION OF THE ADULT RABBIT

Throughout this section frequent reference should be made to Text-fig. 1 B.

In the adult rabbit some ten separate cell columns in the ventral horn of the cervical and upper thoracic regions have been discovered; they are as follows:

Columns 1 *and* 2. These two columns will be considered together because of their close relation throughout the region under consideration. In the first cervical segment columns 1 and 2 appear just caudal to the hypoglossal nucleus at the ventro-medial edge of the ventral horn. Column 1 lies just dorsal and medial to column 2 which is a ventro-medial column throughout the greater part of its length. These two columns retain this arrangement until they reach the upper part of the eighth cervical segment where they fuse into a single column lying in a position intermediate to that taken up by the two separate columns in other regions. Caudal to this, till the upper part of the second thoracic segment is reached, the two columns are irregularly fused. In the upper part of the second thoracic segment they separate; column 1 takes up a ventro-medial position and column 2 an intermediate ventral position. Both these columns are made up of a small number of cells which, in transverse section, appear as typical multipolar cells of the ventral horn. It is usual to find not more than four or five cells at the most in each column in any one section, and the columns do not differ significantly in size from one level to another.

Column 3. In the first cervical segment this column is situated just lateral to column 1 and dorsal to column 2. It retains this arrangement throughout the first and second cervical segments. It then passes abruptly in a lateral direction to reach the medial edge of the lateral funiculus in the dorsal part of the ventral horn in the third cervical segment. This abrupt translation is not readily followed in the toluidin blue sections and is only clearly visible in the younger stages where the columns are more compact. However, in pyridin-silver preparations the two parts of the column are seen to be continuous, not only as a cellular band but also by virtue of the atypical course of their processes. Both these parts of the column send their fibres in a dorsal direction to join the spinal accessory nerve. Traced further caudally, the column passes medialwards to fuse with column 5 and takes up a central position in the caudal part of the fourth cervical segment. It is not known whether column 5 extends as far as the sixth cervical segment, in combination with column 3, but fibres can be traced into the spinal accessory nerve from the caudal extremity of this column, denoting the presence of column 3.

Column 4. This column appears in the caudal part of the third cervical segment lying at the lateral edge of the ventral horn between column 2

ventrally and column 3 dorsally. Caudal to the third cervical segment the column remains in the same situation extending into the upper thoracic spinal cord. In the caudal part of the eighth cervical segment it is closely related to and often fused with column 8. In the upper part of the first thoracic segment it fuses with column 9, mainly due to the fact that column 4 is sweeping medially into the narrower ventral horn of the upper thoracic segments and so impinges on the ventrally situated column 9. Throughout, column 4 is placed ventro-laterally. In the upper cervical region column 4 is of the same size as columns 1 and 2, but, as it sweeps into the laterally expanding ventral horn in the region of the fourth and fifth cervical segments, the number of cells which it contains increases and remains large until the middle of the first thoracic segment where it decreases in size as it sweeps medialwards.

Column 5. In the cephalic part of the fourth cervical segment column 5 is situated centrally in the ventral horn dorso-medial to column 4 and ventro-medial to column 3. It extends caudally in this situation to the lower part of the fourth cervical segment where column 3, passing medially, fuses with it. The fused columns pass caudally together as far as the upper part of the sixth cervical segment where they disappear. It is not possible to say how far column 5 extends caudal to its fusion with column 3, though it is known that cells of column 3 are found throughout the fused part. This column does not show any marked difference in size at various levels, consisting of some five cells in most sections.

Column 6. Column 6 appears in the cephalic part of the fifth cervical segment in the same position as that taken up by column 3 in the third and fourth cervical segments, that is, in the dorso-lateral part of the ventral horn dorsal to column 4. It retains this position throughout the fifth and cephalic parts of the sixth cervical segments, being displaced medially by the appearance of column 7 lateral and dorsal to it. In this new central position it passes caudally to fade out in the lower part of the eighth cervical segment in a position almost directly caudal but somewhat ventral to that taken up by the fused parts of columns 3 and 5. This column increases rapidly in size after its first appearance, reaching a maximum in the sixth cervical segment and decreasing in size again with the appearance of column 7 which seems to be split off from it. Throughout the seventh and upper parts of the eighth cervical segments it remains large, containing more cells than any of the previously described columns. It disappears rapidly in the lower part of the eighth cervical segment.

Column 7. This column appears in the caudal part of the sixth cervical segment by fission from the dorso-lateral aspect of column 6. Traced in a caudal direction, it is displaced dorsally by the appearance of column 8 between it and column 4 ventrally. It is continued caudally in this new situation into the caudal part of the first thoracic segment where it ends. This column does not show any great variation in size and contains about five cells when seen in transverse section.

Column 8. Column 8 appears in the eighth cervical segment at the lateral edge of the ventral horn, dorsal to column 4, lateral to column 6, and ventral to column 7. It is continued caudally in this position till it ends in the lower portion of the first thoracic segment. In the lower part of the eighth cervical and upper part of the first thoracic segments it tends to fuse ventrally with column 4. This column forms the most lateral edge of the ventral horn at the point where it extends furthest laterally in the cervical enlargement; it contains some eight to ten cells in transverse section and does not show any great changes in size except at either end where it fades out gradually.

Column 9. This is a short, ventrally situated, column passing from an intermediate ventral position in the ventral horn of the eighth cervical segment to fuse with the medial aspect of column 4 in the caudal part of the first thoracic segment, where this column inclines medially to enter the lateral part of the narrower thoracic ventral horn.

Column 10. This column appears dorsal to column 7 in the lower part of the eighth cervical segment and here constitutes the most dorsally situated cell column of the ventral horn in the cervical enlargement. It extends further caudally than columns 6, 7 or 8, entering into the cephalic two-thirds of the second thoracic segment where it ends in close proximity to the cephalic end of the intermedio-lateral cell column. Column 10, at first small, rapidly enlarges, reaching a maximum in the middle of the first thoracic segment where transverse sections frequently show between five and ten cells.

Caudal to the end of column 10, there appears the intermedio-lateral cell column which, together with columns 1, 2 and 4, makes up the columnar arrangement of the upper thoracic segments.

THE CELL COLUMNS IN EMBRYOS AND FOETUSES OF VARIOUS AGES

Sections of a 6 mm. rabbit embryo show that the cervical spinal cord consists of a closed tube made up of a layer of germinal cells thickened in the ventral horn region, but without any sign of differentiation of the cells or any cell columns.

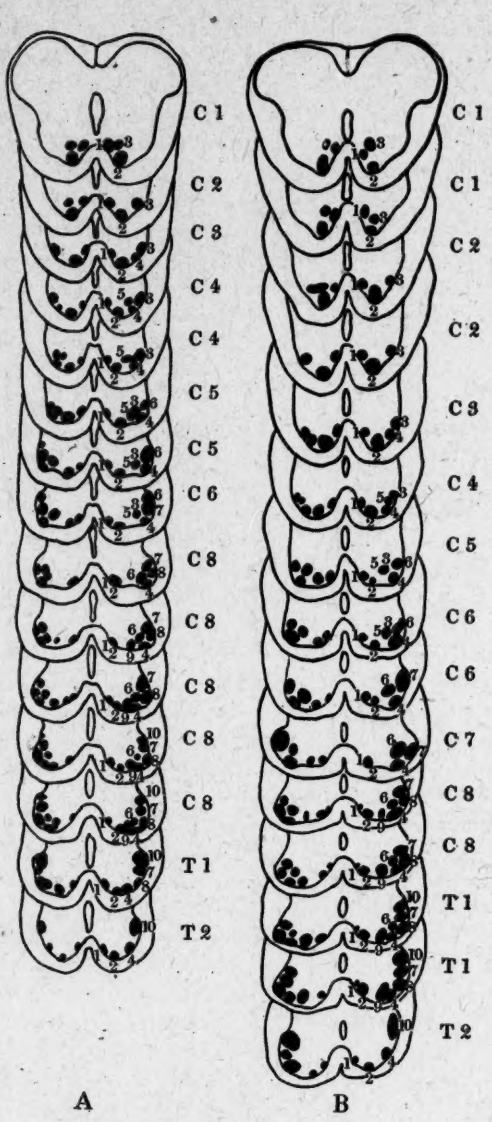
By the time that the 8 mm. stage has been reached a definite ventral horn has been formed containing cells which stain more lightly than the parent germinal cells (Pl. 1, fig. 1). These cells are arranged in a dense mass within which no division into cell columns can be seen in any plane of section. In the 11 mm. rabbit embryo, however, the first signs of cell groupings are visible and they take the form of an increased density of the undifferentiated neuroblasts into definite longitudinally arranged columns. These columns are difficult to interpret because of the density of the cells in the ventral horn and the undifferentiated nature of the cells forming the columns. In sagittal section (Pl. 2, fig. 3) it is impossible to differentiate the ventral horn cells into particular groups with any degree of certainty. In the coronal sections of the cervical

enlargement, however, a laterally placed group, which appears homogeneous in sagittal section, can be differentiated from a centrally placed group (Pl. 3, fig. 2). In this plane of section no further groups are visible and it is in transverse section that the greatest degree of differentiation is demonstrable. Columns 1 and 2 are present but fused with each other throughout the greater part of their length. The lateral part of the ventral horn lodges a large group extending dorsally as far as the dorso-lateral part of the ventral horn (Pl. 1, fig. 2). This large column, which is particularly deep in the caudal part of the cervical enlargement, appears to be split in some sections into more than one column. However, since this group shows no clear divisions in sagittal sections, the above appearance is considered to be due to the passage of fibres and blood vessels through it. This is borne out by the inconsistent nature of the division when compared with older embryos. This group probably represents all the cell columns of the lateral part of the ventral horn in an undifferentiated state.

In the cephalic part of the cervical spinal cord, columns 3, 4 and 5 are present as separate entities, except in the region of fusion between columns 3 and 5. Traced caudally, column 4 fuses with the general lateral mass in the fifth cervical segment. Column 3 deserves special notice because the cells which it contains have reached a greater degree of histological differentiation than the cells of any other group at this stage. These cells are less elongated and contain a more darkly staining and definite Nissl substance in a greater volume of cytoplasm than in the other undifferentiated neuroblasts.

There is some suggestion of a separation of column 6 in the caudal part of the enlargement but this is not so definite as the separation of column 5. It is not possible to differentiate column 9 from the mass of cells forming the most ventral part of the ventral horn in the eighth cervical segment.

By the 15 mm. stage (Pl. 1, fig. 3; Pl. 2, fig. 4) a further degree of differentiation has been reached, not only in the cell columns but also in the cells forming the columns, many of which resemble the cells seen in column 3 of the 11 mm. stage. In the first three cervical segments the arrangement of the columns is similar to that in the 11 mm. embryo except that columns 1 and 2 are more readily separable in this region though they fuse again in the fourth cervical segment and are not separable till the first thoracic segment is reached. Columns 3 and 5 are arranged as in the adult. Column 6 is distinct throughout its length and has its adult relations. Column 4 is also distinct throughout the greater part of its length except for occasional fusions with adjacent columns, especially in the eight cervical segment where it is fused with column 9 which is seen at this stage as a thickening of column 4 and only separable from the latter in its most cephalic part. Columns 7 and 8 are irregularly fused but are definitely separated from column 4 ventrally and column 6 medially throughout the greater part of their length. Column 7 is particularly large in the last cervical and first thoracic segments, but column 10 is not visible as a separate entity except in so far as the dorsal part of column 7 appears to extend further



Text-figs. 1A, B. Diagrams based on serial transverse sections of the cervical enlargement of the spinal cord in 26 and 41 mm. rabbit embryos respectively. 1A, ×36; 1B, ×30.

caudally than in the adult. This appears to be the caudal part of column 10 fused further cephalad with column 7.

In the 19 mm. stage all the columns described in the adult rabbit are visible as separate entities, though considerable fusions occur in various regions between these columns (Pl. 1, fig. 4; Pl. 3, fig. 1). These fusions do not appear to be constant and so are not considered worthy of detailed description. Column 7 is now separated from columns 8 and 10, column 9 is separate from column 4, except in its caudal part, columns 1 and 2 show a smaller degree of fusion which extends only from the seventh cervical to the first thoracic segments.

From this stage no significant changes in the arrangement of the cell columns have been seen and all subsequent changes are in the histological appearance and arrangement of the cells themselves within the columns.

In the 26 mm. stage (Text-fig. 1A; Pl. 1, fig. 5) the cells show as very discrete clumps histologically differentiated from the cells of the other stages and from the indifferent cells of the ventral horn not situated within the cell columns. The columns at this stage are more definite than at any previous or succeeding stage, for though a great number of undifferentiated cells, not appearing in the older stages and especially in the adult spinal cords, still exist between the columns, the density of cell grouping in these columns is very great. In this stage columns 1 and 2 show an even smaller degree of fusion which extends only through the eighth cervical and upper part of the first thoracic segments, and the degree of fusion seen between the various columns of the lateral part of the ventral horn is very much reduced, not exceeding that found in the adult.

In the 41 mm. rabbit embryo (Text-fig. 1B; Pl. 2, fig. 1), the cell columns are very definite but do not appear so compact as in the 26 mm. stage when viewed in transverse section. This decrease in the solidity of the columns increases steadily throughout foetal, and rapidly throughout postnatal, life, till, in the adult rabbit, cell columns are with difficulty separable from each other on account of the scattered nature of their cells (Pl. 2, fig. 2). It seems very probable that this scattering of the cells is conditioned by the growth of the spinal cord which continues late into postnatal development, the postnatal growth of the spinal cord in the rabbit being much greater than in Man, for in a full grown rabbit the spinal cord reaches into the upper part of the sacrum. In this connexion it is to be noted that the area of the grey matter in the eighth cervical segment as seen in transverse section increases rapidly after birth. It is in this stage that the thinning out of the columns is most marked and the growth in length of the spinal cord is most rapid.

In summary, the cell columns of the cervical enlargement of the spinal cord of the rabbit develop very rapidly, reaching their adult arrangement, with the exception of some minor changes, by 16½ days after copulation and their most compact stage 2½ days later in the 26 mm. rabbit embryo, having commenced their development in the 11 mm. stage, that is on the 14th day after copulation.

This very rapid development of the columns and the undifferentiated nature of the cells involved make it difficult to determine the exact order of development of the cell columns, but, in general, the columns in the lateral part of the ventral horn appear to develop later than those on its medial aspect out of a common mass of cells whose differentiation passes in a ventro-dorsal direction; that is, the most ventral columns of the lateral part of the ventral horn develop out of the common mass before the most dorsally situated columns (Pl. 1, figs. 1–4).

This description of the developing cell columns follows closely that on the white rat which Angulo y González (1940) published after this work was completed. It is important to note that this development is very similar in the two types of animals studied, even though the total number of columns present is not the same.

LOCALIZATION

In order to determine whether any correlation can be found between the cell columns seen in the spinal cord and the peripheral groups of muscles, the following series of experiments was carried out on adult rabbits:

Table 2

Nerves cut	Type of section	Position	Duration of experiment/ days
Median and ulnar	Crushing and cutting	Wrist	13
Median, ulnar and radial	„	Elbow	13
Median, ulnar, radial and musculo-cutaneous	Simple cutting	Axilla	21
Median and ulnar	Violent rupture	Wrist	13
Median and ulnar	„	„	7

All these experiments were performed under aseptic conditions with a view to showing chromatolytic changes in the cells of the various columns in the spinal cord. The animals were killed with an overdose of chloroform and injected with 10% formalin which is a satisfactory fixative for adult rabbit material to be stained with toluidin blue or gallocyanin. The cervical enlargement from the fifth cervical to the second thoracic segments inclusive was removed and sectioned.

Study of these sections showed that none of the cells in the cervical region displayed the typical reaction which has been designated chromatolysis. Certain of the cells on the operated side showed an arrangement of the Nissl substance which was not visible on the unoperated side, consisting of a grouping of the Nissl substance as a more homogeneous ring around the centrally placed nucleus with a clearer zone of cytoplasm between this and the cell membrane. Nissl substance was absent in the dendrites. This was the greatest change observed, and many others which appeared slightly different from the normal were not readily distinguishable from those on the unoperated side. In the case of section of the median and ulnar nerves these changed cells were located in the most caudal and dorsal cell column of the enlargement (column 10); in the case of section at the elbow, cells had also undergone a change in column 7.

Table 3 shows a similar but more extensive series of experiments carried out on adult mice.

These animals were killed with chloroform and either the spinal cord was removed and fixed in acetic-alcohol or Heidenhain's Susa, or the whole spinal column was removed and fixed in Jenkin's fixing decalcifying fluid. The cervical regions, fifth cervical to second or third thoracic segments, were imbedded with the methylbenzoate-celloidin-paraffin technique and serially sectioned. The sections were stained either with toluidin blue or gallocyanin.

There were no cells visible in any of these spinal cords showing definite signs of the chromatolytic reaction and, though there were many which did not appear quite normal, none of them was sufficiently different from those on the uninjured side to allow of a definite analysis of the changed cells. The commonest change took the form of a decreased granularity of the Nissl

Table 3

Nerves cut	Type of section	Position of section	Duration of experiment
Median and ulnar	Simple cutting	Elbow	5 days
"	"	"	9 "
"	"	"	13 "
"	"	"	21 "
"	Section with cautery	"	5 "
"	"	"	8 "
"	"	"	9 "
"	"	"	12 "
"	"	"	21 "
"	Violent rupture	"	8 hours
"	"	"	18 "
"	"	"	28 "
"	"	"	38 "
"	"	"	48 "
"	"	"	3 days
"	"	"	4 "
"	"	"	5 "
"	"	"	6 "
"	"	"	9 "
"	"	"	12 "
"	"	"	21 "
"	"	"	102 "

substance in the cell cytoplasm which thus stained more homogeneously but always with a greater density around the nucleus, though in no case was the clear zone seen in the rabbit visible in any cell in the mouse spinal cord. It is of interest that cells showing these slight changes were present in the most caudal and dorsal cell columns of the cervical enlargement in both animals.

These results suggest that chromatolysis is far from being a satisfactory method, at least in the study of the origin of spinal nerves, since, even if some cells are induced to undergo chromatolysis, many others may only show the changes described above or may even remain unchanged and so upset any attempt to determine the central origin of the cut nerve. Several other investigators have found difficulties in producing chromatolysis. Prof. Le Gros Clark (personal communication) has found considerable difficulty in deter-

mining the distribution of chromatolysed cells in the lumbo-sacral spinal cord of monkeys after section of the sciatic nerve. De Neef (1901) found that anything but violent rupture was ineffectual in producing chromatolysis, and Marinesco (1898*a*) described a series of degrees of chromatolysis depending on the intensity of damage to the nerve cell.

However, despite the unsatisfactory nature of these results, in all the rabbits in which section of the median and ulnar nerves at the wrist was carried out the cell changes were confined to one cell column only, column 10, extending from the lower part of the eighth cervical segment to the upper part of the second thoracic segment and situated further dorsally than any other cell column in the enlargement. It seems probable that the cells lying in this column send their axons to the muscles of the hand. This result is borne out by the researches of de Neef (1901) who investigated the cell columns in the cervical and lumbo-sacral enlargements of the rabbit and dog. His description of the columns in the cervical enlargement of the rabbit does not agree in all respects with that given above. However, certain columns are common to the descriptions and one of these is column 10 which de Neef calls column D and shows extending from the eighth cervical to the lower part of the first thoracic segment. By successful chromatolytic experiments de Neef found that this column D contained the cells supplying the hand muscles. Similar findings in regard to column 7 (column C of de Neef) suggest that it contains the cells of origin of the fibres supplying the muscles of the forearm. However, it has been impossible to form any direct opinion on the functional relations of the other cell columns, though on the basis of the findings of de Neef, Bok, v. Gehuchten and other authors it seems likely that the more ventrally situated cell columns supply the more proximal segments of the limb.

It is of great importance to note here that van Gehuchten and de Neef carried out a very comprehensive series of experiments in order to produce chromatolysis. This they were unable to do except by violent rupture of the nerves concerned. This latter technique has been tried here without success, so it seems that chromatolysis as an experimental method has not the value usually ascribed to it, at least in the spinal nerves of the animals described above.

DEVELOPMENT OF CELL PROCESSES IN THE VENTRAL NERVE ROOTS OF THE CERVICAL REGION OF THE RABBIT

The first cell processes to develop are very fine, faintly argentophil, protoplasmic strands connecting the ventral region of the spinal cord of the 6 mm. rabbit embryo to the surrounding mesenchyme. It is impossible to follow these fibres further than the most proximal mesenchyme and their junction with another group of fibrils arising from the dorsal root ganglia; beyond this point, although the fibres are not visible, the mesenchymal cells are orientated along the axis of these protoplasmic strands. In the 8 mm. rabbit embryo a much greater density of staining of the roots is visible, ventral and dorsal roots are

fused, and they pass ventro-laterally towards the developing limb bud, stopping abruptly at its proximal end where the various segmental nerves meet to form a plexus. There is a short posterior primary ramus which only penetrates a short distance into the dorsal musculature. At this stage there is no neuro-muscular contact between the ventral horn cells and either the developing limb or dorsal musculature.

In the 11 mm. stage the trunks of the nerves have grown peripherally into the limb bud as far as the future wrist joint region and the posterior primary ramus has extended into the dorsal musculature. With a closer study of the nerve fibres in the limb it is found that, though the trunks of the nerves extend far into the limb, only the most proximal muscles contain any such fibres ramifying among them in close relation to the developing myoblasts; thus it appears that a few neuro-muscular units may have been established between the ventral horn cells of the spinal cord and the developing muscles of the trunk and shoulder regions, though neuro-muscular endings are not visible.

In the 15·5 mm. embryo the trunks of the nerves have extended into the distal extremity of the forelimb bud, many more nerve fibres can be found ramifying in the dorsal and shoulder musculature, and a few are found leaving the trunks of the nerves to enter the arm and forearm musculature. In this stage therefore a closer relation between the nerves and muscles of the trunk and shoulder regions has been established, and a few new connexions with the arm and forearm musculature have been attained.

In the 19 mm. embryo fine cell processes can be found leaving the trunks of the nerves throughout the limb (Pl. 3, figs. 4, 5). These are most marked in the shoulder muscles and least in the muscles of the hand. It seems possible that at this stage neuro-muscular connexions have been established with some muscle fibres of all the segments of the limb and certainly with the dorsal musculature, which in this stage shows reflex contraction on stimulation of the snout, though no neuro-muscular endings have been observed.

The 26 mm. rabbit embryo shows a considerable increase in the number of fine nerve fibres ramifying among the muscles of the forelimb. This increase is especially marked in the muscles of the hand when compared with the previous stage. Definite neuro-muscular endings have appeared in the trunk and proximal limb muscles (Pl. 3, fig. 6), and correlated with these is the appearance for the first time of reflex motility in the limb. With the mother under urethane anaesthesia, movements of the trunk, shoulder and arm muscles were seen on opening the amnion of these foetuses. They did not persist, but could be elicited on stimulation of the snout with a weak faradic current. This first appearance of motility is rather later than that described by Pankratz (1939) who found the earliest movements at 16½–17 days, but since no intermediate stages have been studied here it seems probable that the earliest movements were just missed, for those of the 26 mm. embryo are quite well developed.

DEVELOPMENT OF THE NISSL SUBSTANCE

The first cells to differentiate from the parent germinal epithelium appear in the 8 mm. stage. These cells stain lightly, the nucleus being clear and containing a well-formed nucleolus. The two apical spicules of cytoplasm stain a faint uniform colour with toluidin blue but show a faintly granular appearance under the high power.

In the 11 mm. stage two distinct types of cell are seen, the first consisting of those cells developing within the column of the ventral horn, and the second of those lying in the ventral horn but not included in the columns. The latter are similar to the cells of the previous stage but the former stain more darkly with toluidin blue, due to the increased staining properties of the nucleus which shows a dense chromatin network and a darkly staining nuclear membrane. The cytoplasm is similar to that in the cells of the 8 mm. rabbit embryo.

From this stage onwards the nucleus gradually loses its strongly staining properties and the cytoplasm stains with increasing intensity until, in the 19 mm. embryo, the nucleus is again quite clear and the cytoplasm stains densely and is finely granular.

In the 26 mm. embryo true Nissl granules appear for the first time around the periphery of the cells situated in the columns of the ventral horn (Pl. 3, fig. 3). These consist of large aggregations of the finely granular substance, diffused throughout the cytoplasm in the earlier stages. Around the nucleus there is still a large quantity of the diffuse substance though the cytoplasm stains less darkly here than at the periphery. The further development of the Nissl bodies consists of the decrease in density of staining of the perinuclear cytoplasm and the gradual extension of these bodies into this region of the cytoplasm where they appear in the 80 mm. rabbit foetus, though it is not till 11 days after birth that all traces of the fine granular deposit are lost and the Nissl bodies are free in a clear cytoplasm.

In regard to the arrangement of the Nissl granules it is interesting to note that they are present to a greater degree in the more ventral cell columns than in those more dorsally situated (Pl. 3, fig. 3, cf. *a*, *b*). This may be correlated with the greater degree of activity in the proximal rather than the distal segments of the limb at this stage, for no movements attributable to forearm or hand musculature are visible in the 26 mm. embryo.

DISCUSSION

There are four distinct stages in the development of the cells in the ventral horn of the spinal cord and the groupings which they form:

(1) A stage of differentiation of the cells in the ventral horn from the germinal epithelium. This consists of a decrease in their density of staining and the appearance of a bipolar shape. In this stage the first cell processes make their appearance. These are at first faintly argentophil protoplasmic strands extending a short distance into the surrounding mesenchyme, but later they

stain more strongly and are traceable to the base of the limb bud, and towards the dorsal musculature in the form of a rudimentary posterior primary ramus. No cell groupings are visible, but the cells are orientated with their long axes directed towards the point of exit of the ventral root.

(2) This stage is marked by the appearance of cell groupings or columns (Pl. 1, fig. 2) and begins with the entry of the cell processes into the limb and dorsal musculature, but as yet no functional or anatomical connexion has been established between the muscles and nerves. The cell columns form two distinct groups. The first is ventro-medial and is found throughout the greater part of the spinal cord; the second, which is lateral in position and extends dorsally for a variable distance, is only present in that part of the cord supplying the limb musculature, and represents the mass of cells out of which the various cell columns of the lateral part of the ventral horn will develop. The cells are similar to those of the last stage but those in the columns tend to show a more darkly staining nucleus and cytoplasm, a considerable quantity of Nissl substance being freely scattered in the cytoplasm without any sign of Nissl granules.

(3) This stage is marked by the splitting of the lateral cell mass into subsidiary columns (Pl. 1, figs. 2–4) commencing with the separation of its most ventral part and terminating with the final formation of a separate columnar form in its most dorsal cells. Peripherally the cell processes enter into close relation with the various muscles of the limb and trunk. This process proceeds proximo-distally, and as each segment of the limb is progressively innervated a further cell column separates from the main lateral mass of cells in the ventral horn of the spinal cord, in such a way that at the end of this stage all the cell columns present in the adult are visible. Though it has been impossible to show any definite nerve terminals on the muscles at this early stage, a functional connexion between muscle and nerve must be present at least in the trunk by the 19 mm. stage when slight reflex activity of these muscles can be obtained on stimulation of the snout. The cells of this stage are very similar in shape to those of the preceding stage though now the cytoplasm stains a denser homogeneous blue and the nuclei are clear.

(4) During this last stage definite reflex and spontaneous movements which appear first in the proximal part of the limb were observed, but there was no appreciable change in the arrangement of the cell columns. The Nissl substance within the cells shows an interesting change, being bunched together in blocks or granules around the periphery of the cells. These granules are at first most marked in the ventrally situated cell column of the lateral group (Pl. 3, fig. 3) which is presumably the column associated with the innervation of the proximal part of the limb, the first part to become active. Several authors (Flesch, 1884; Hodge, 1892; Mann, 1894; Nissl, 1896; van Durme, 1901; Carlson, 1902; Dolley, 1909; Malone, 1913; Kocher, 1916; Cowdry, 1924; Hopkins, 1924; Clark, 1926) have described differences in the arrangement of the Nissl substance in nerve cells after various fixatives and degrees of activity

at the time of fixation. It is therefore interesting to note that the Nissl granules appear at the time when the cells first show signs of functional activity.

Superimposed upon this general arrangement of the development of the cell columns is a tendency for their more cephalic members to appear first, thus showing a general cephalo-caudal differentiation. The last column to appear, therefore, is the most caudal and dorsal of the lateral group.

Correlated with the anatomical changes just described are three different functional periods in the development of the cells of the ventral horn:

(1) A period during which the cells are sending out their processes towards the muscles which they are to supply. In this stage all the cells, whatever their destination may be, are functionally alike in their growth processes and so might be expected to form a single undifferentiated aggregation.

(2) A period during which the cells consolidate their connexions with the peripheral muscle fibres. Such a period must of necessity affect the proximal neuro-muscular connexions first and the distal connexions last, but in each segment of the limb all connexions occur almost simultaneously. So, for example, while one group of cells is connecting with the proximal muscles of the limb, others passing to more distal parts are as yet still in stage one. Such an arrangement produces two groups of functionally and histologically different cells within the original cell column. It is this difference which is regarded as the factor in the splitting of the main cell mass into successive subsidiary groups, each related to a more distal segment of the limb in order of appearance.

(3) A period of functional activity of the nerve cells which leads to active contraction of the muscle fibres. This change results in a histological rearrangement of the Nissl substance (whose adult arrangement is not reached until postnatal activity has been in progress for some time) and the appearance of definite neuro-muscular endings, but there is no change in the arrangement of the cell columns which are, apart from their greater density, adult in form at the end of stage two.

With regard to the localization of function within the cell columns of the spinal cord, direct evidence concerning the connexions of the cells is lacking owing to the unsatisfactory nature of the chromatolysis reaction in the cells of origin of spinal nerves, but certain facts concerning the development of the cell columns throw light on their function:

(1) The cell columns are developed at a time when no functional activity in the form of muscle movements has been noted, so it seems certain that they cannot be considered as the result of the relation of the cells to peripherally functioning groups of muscles.

(2) Several cell columns have already appeared before all the muscles of the limb have been innervated, thus suggesting that each cell column does not supply all the muscles of the same functional activity throughout the limb.

Combined with the fact that one cell column only has been affected in

lesions denervating the muscles of the hand in the rabbit, and that further spread into a second column has been noted on denervation of the forearm and hand, the two points mentioned above bear out the findings of de Neef (1901), Bok (1928) and other authors, localizing segments of the limbs in specific cell columns.

The cell columns described above cannot be considered as aggregations of cells formed as a result of the similarity of function of the muscles which they supply, as has been suggested by Goering (1928) in the white rat; but must be considered as related to specific segments of the limb, both embryologically and functionally. This will mean that within any one cell column there exist side by side cells supplying flexors, extensors and rotators of the segment innervated by that column. It seems likely that as these cells differ more functionally from each other the cell columns will not tend to become more definite by closer and closer aggregation of their component parts, as would probably be the case if all the cells were exactly similar in function, but the cell columns might even tend to become less distinct and broken up into several parts. Such a process of de-differentiation of the cell columns has been shown to take place, starting with the beginning of activity in the foetus and receiving added impetus during postnatal development, with the result that cell columns in the spinal cord of adult rabbits and in all other mature animals yet studied are made up of very diffuse cell groupings which are difficult to determine in comparison with the same columns in the late embryonic stages (cf. Pl. 1, fig. 5; Pl. 2, fig. 2). This de-differentiation may not be an active process and may be related to the growth of the intercellular substance of the grey matter in the spinal cord, but it shows that there is no functional tendency for the cells within the columns to aggregate.

If the interpretation of the mechanism of development of the cell columns given above is correct, then their configuration will be dependent upon the arrangement of the segments of the limb supplied. Thus mammals showing a similar arrangement of the limb might be expected to show a like arrangement of the cell columns, though variations in the number of cells within the columns would be expected in relation to the increased or decreased amount of muscle supplied (Shorey, 1909; Hamburger, 1934). That such a similarity exists, despite functional differences, is evident from a comparison of the cell columns in the cervical and lumbo-sacral enlargements of the spinal cord of man (v. Gehuchten & de Neef, 1900, Jacobsohn, 1908, Romanes, 1941) which are practically the same, the only marked difference lying in the relative size of the various columns. That this similarity can be extended to other mammals with like limbs seems certain from the findings of de Neef and other workers, and the preliminary researches of the author into the comparative anatomy of these cell columns in a variety of mammals. That the converse of this is true is well known and it is only necessary to mention two examples here. The first is the condition of the spinal cord in the porpoise (Hepburn & Waterston, 1904) where reduction of the limbs and cell columns is present, and the second,

reported by Yu-Ch'uan Tsang (1939), is the further differentiation in a polydactylous strain of mice of the most caudal and dorsal cell column of the lumbar enlargement which supplies the foot.

Thus the cell columns of the spinal cord undergo changes in their development which reflect and coincide with the proximo-distal differentiation of the various parts of the limb musculature and its associated nerves.

SUMMARY

The adult anatomy and development of the motor cell columns in the ventral horn of the cervical enlargement in the rabbit are described in relation to the developing peripheral structures. Four chief stages have been noted:

(1) A thickening in the ventral part of the germinal epithelium of the neural tube to form a ventral horn.

(2) The outgrowth of processes from the ventral horn cells, the latter grouping themselves together to form longitudinal aggregations.

(3) The secondary splitting of these aggregations into subsidiary columns of cells coinciding with their connexion to the muscles which they supply, but prior to reflex activity.

(4) The appearance of coordinated neuro-muscular activity leading to contraction of the peripheral muscles. In this stage true Nissl granules appear in the cytoplasm of the ventral horn cells.

The establishment of neuro-muscular connexions, which are distinct only in the last stage, proceeds peripherally from segment to segment along the limb, a new column appearing as each segment is innervated. Thus the columns are embryologically related to segments of the limb, and there is evidence in the adult to suggest that such a functional relation exists, though it is not complete owing to the unsatisfactory nature of the chromatolytic reaction.

I wish to express my thanks to Prof. Harris for his help and encouragement throughout this investigation.

REFERENCES

Angulo y González, A. W. (1927). *J. comp. Neurol.* **43**, 115.
—— (1940). *J. comp. Neurol.* **73**, 469.
Bok, S. T. (1928). W. v. Möllendorf's *Handbuch der Mikroskopischen Anatomie des Menschen*, **4/1**, 478.
Bruce, A. (1901). *A Topographical Atlas of the Spinal Cord.* London.
Carlson, A. J. (1902). *Amer. J. Anat.* **2**, 341.
Clark, S. L. (1926). *J. comp. Neurol.* **41**, 423.
Cowdry, E. V. (1924). *General Cytology.* Chicago.
Dolley, D. H. (1909). *Amer. J. Physiol.* **25**, 151.
v. Durme, P. (1901). *Névraxe*, **2**, 115.
Flesch (1884). Quoted from v. Gehuchten, *Système Nerveux.* Louvain.
Frazer, J. E. (1931). *Manual of Embryology.* London.
v. Gehuchten, A. & de Buck (1898). *J. Neurol., Brux.* Quoted from *Système Nerveux.*

v. Gehuchten, A. & de Neef, C. (1900). *Névraxe*, **1**, 201.
v. Gehuchten, A. & Nelis (1899). *J. Neurol., Brux.* Quoted from *Système Nerveux.*
Goering, J. H. (1928). *J. comp. Neurol.* **46**, 125.
Hamburger, V. (1934). *J. exp. Zool.* **68**, 449.
Hammond, G. M. (1894). *N.Y. med. J.* **59**, 15. Quoted from Angulo (1927).
Hepburn, D. & Waterston, D. (1904). *J. Anat., Lond.*, **38**, 105.
Hodge, C. F. (1892). *J. Morphol.* **7**, 95.
Hopkins, R. E. (1924). *Anat. Rec.* **28**, 157.
Jacobsohn, L. (1908). *Ueber die Kerne des menschlichen Rückenmarks.* Berlin.
Kreyssig (1885). *Virchows Arch.* **102**, 286.
Kocher, R. A. (1916). *J. comp. Neurol.* **26**, 341.
Malone, E. F. (1913). *Amer. J. Anat.*, **15**, 121.
Mann, G. (1894). *J. Anat. Physiol.* **29**, 100.
Marinesco, G. (1898*a*). *Rev. Neurol.* **6**, 463.
—— (1898*b*). *Pr. méd.* p. 201. Both quoted from *Système Nerveux.*
de Neef, C. (1901). *Névraxe*, **2**, 71.
Nissl, F. (1896). *Z. Psychiat.* 1147.
Oberstеiner, H. (1890). *The Anatomy of the Central Nervous Organs*, trans. Alex. Hill.
Onuf, B. (1899). *J. nerv. ment. Dis.*
Pankratz, D. S. (1939). *Anat. Rec.* **73**, Suppl. p. 72.
Romanes, G. J. (1941). *J. Anat., Lond.*, **75**, 145.
Sano, F. (1897). *Premier Congrès International de Neurol., Brux.*, p. 37.
—— (1898). *Ann. Soc. Méd.-Chir. Anvers.* Both quoted from de Neef, C. (1901).
Sass, A. (1889). *Virchows Arch.* **116**, 243.
Schiefferdecker, P. (1874). *Arch. Mikr. Anat.* **10**, 471.
Shorey, M. L. (1909). *J. exp. Zool.* **7**, 25.
Steida, L. (1870). *Studien ueber das Centralnervensystem der Wirbelthiere.* Quoted from de Neef, C. (1901).
Waldeyer, W. (1889). *Das Gorilla Rückenmark.* Berlin.
Yu-Ch'uan Tsang (1939). *J. comp. Neurol.* **70**, 1.

EXPLANATION OF PLATES 1–3

Plate 1

Fig. 1. Transverse section through the ventral horn of the spinal cord of an 8 mm. rabbit embryo in the 8th cervical segment. Stain, toluidin blue. ×400.
Fig. 2. Transverse section through the ventral horn of the spinal cord of an 11 mm. rabbit embryo in the 8th cervical segment. Stain, toluidin blue. ×400.
Fig. 3. Transverse section through the ventral horn of the spinal cord of a 15 mm. rabbit embryo in the 8th cervical segment. Stain, toluidin blue. ×320.
Fig. 4. Transverse section through the 8th cervical segment of the spinal cord in a 19 mm. rabbit embryo. Stain, toluidin blue. ×100.
Fig. 5. Transverse section through the 8th cervical segment of the spinal cord in a 26 mm. rabbit embryo. Stain, toluidin blue. ×60.

Plate 2

Fig. 1. Transverse section through the 8th cervical segment of the spinal cord in a 41 mm. rabbit foetus. Stain, toluidin blue. ×60.
Fig. 2. Transverse section through three cell columns in the ventral horn of the spinal cord of an adult rabbit. These are examples of the densest cell columns seen in the cervical enlargement at this stage. Stain, toluidin blue. ×120.
Fig. 3. Sagittal section through the lateral part of the ventral horn of an 11 mm. rabbit embryo in the 8th cervical segment. Cf. Pl. 1, fig. 2. Stain, toluidin blue. ×400.
Fig. 4. Sagittal section through the lateral part of the ventral horn of a 15 mm. rabbit embryo in the 8th cervical segment. Cf. Pl. 1, fig. 3. Stain, toluidin blue. ×400.

PLATE 3

Fig. 1. Sagittal section through the lateral part of the ventral horn of a 19 mm. rabbit embryo in the region of the 8th cervical and 1st thoracic segments. Cf. Pl. 1, fig. 4. Stain, toluidin blue. ×100.

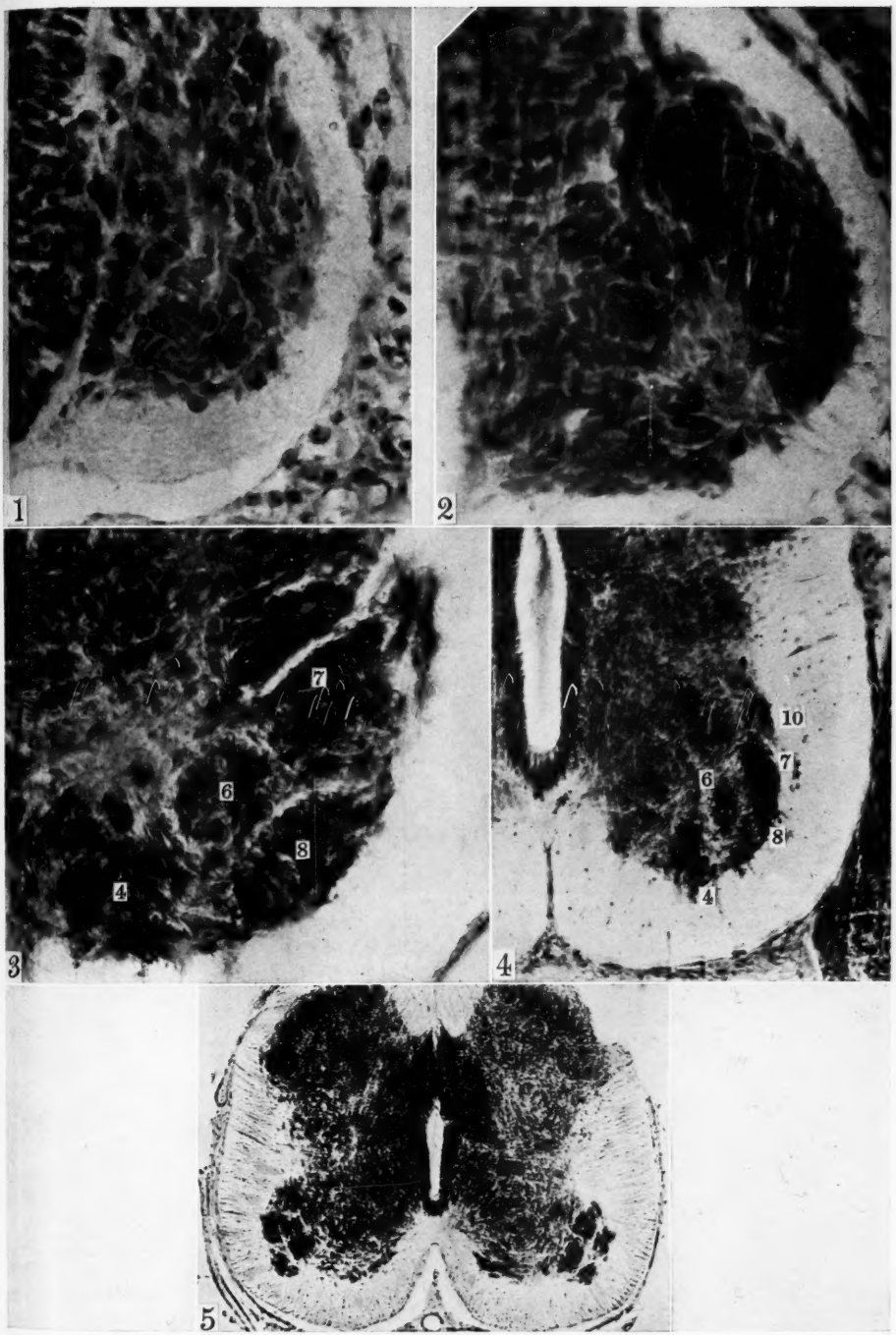
Fig. 2. Coronal section through the upper part of the cervical enlargement in an 11 mm. rabbit embryo. The right half viewed from above is shown. Stain, toluidin blue. ×240.

Fig. 3. Sagittal section through the lateral part of the ventral horn of the cervical region in a 26 mm. rabbit embryo. A, ventral cell column; B, dorsal cell columns. Stain, toluidin blue. ×600.

Fig. 4. Section through the biceps of a 19 mm. rabbit embryo. Stain, pyridin-silver. ×625.

Fig. 5. Longitudinal section through the subscapularis muscle of a 19 mm. rabbit embryo. Stain, pyridin-silver. ×625.

Fig. 6. Longitudinal section through the subscapularis muscle of a 26 mm. rabbit embryo to show neuro-muscular endings. Stain, pyridin-silver. ×425.



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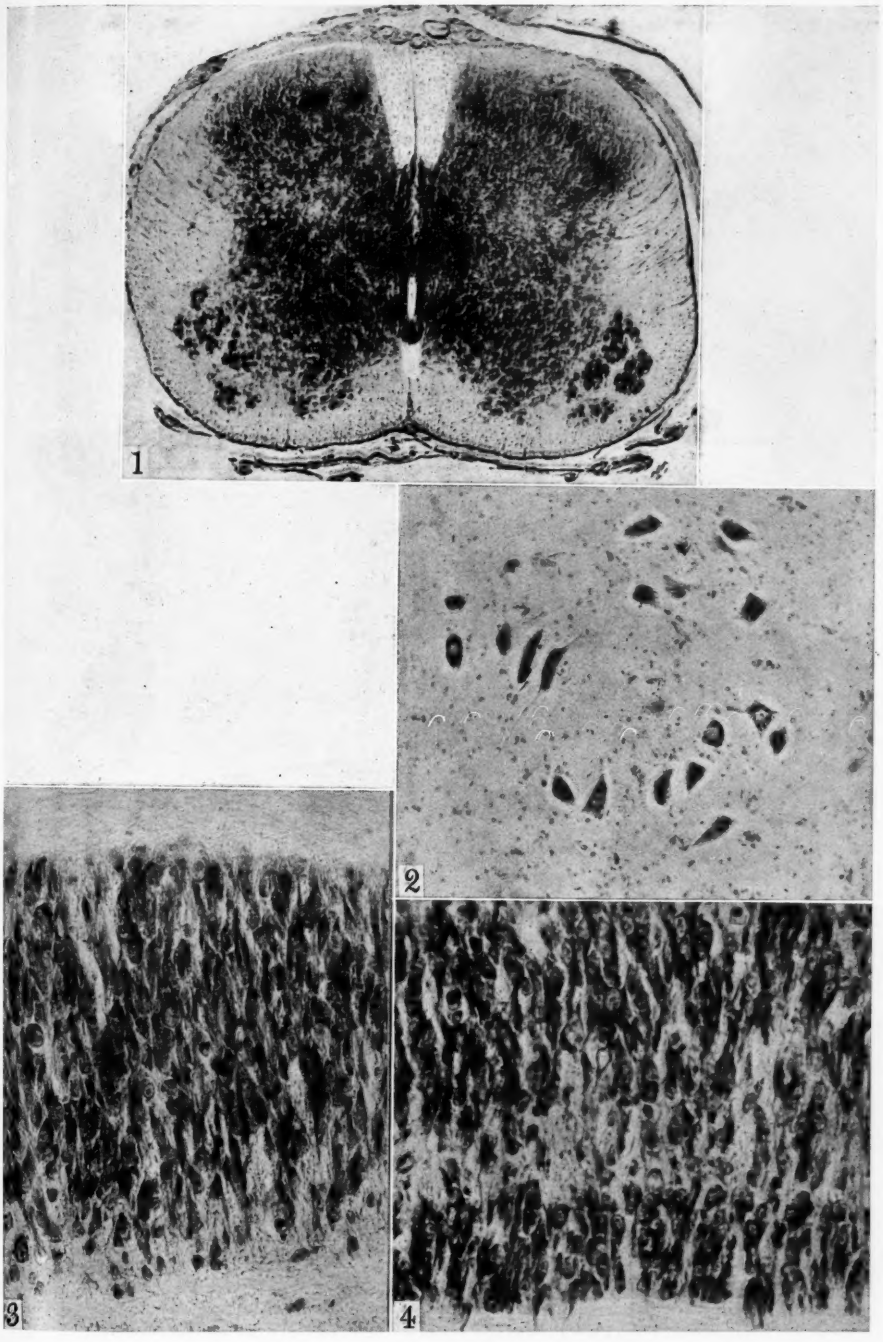

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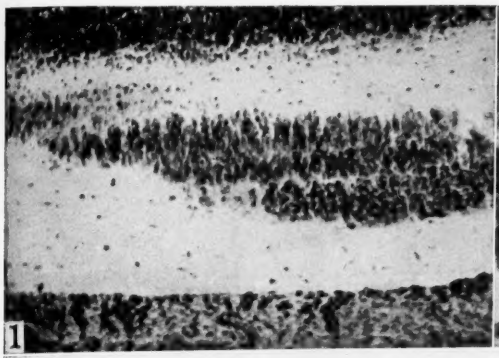

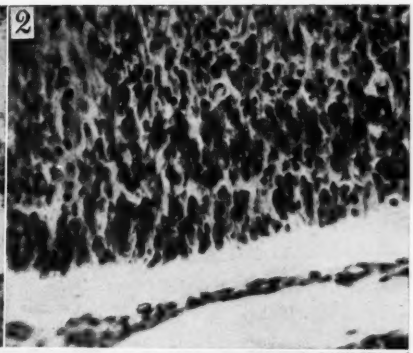

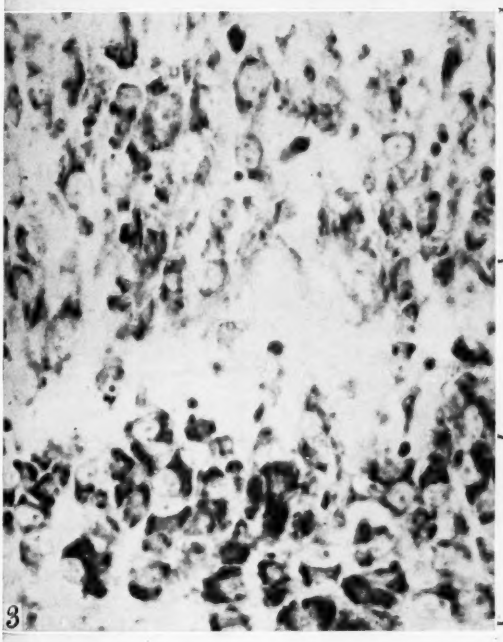

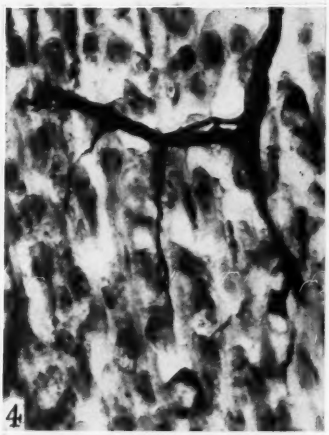

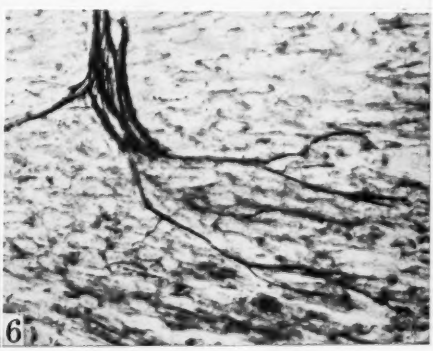

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THE LATERAL GENICULATE BODY IN THE PLATYRRHINE MONKEYS

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THE lateral geniculate body in the Old World monkey, *Macaca mulatta*, shows a regular lamination pattern comprising two large-celled and four small-celled laminae. These layers are sharply defined and, though in certain regions of the nucleus they may be interconnected, they are commonly separated by relatively wide and regular medullary laminae. The cell layers are curved acutely on themselves so as to produce a ventrally directed hilum, and they are related to the fibres of the optic tract in such a way that (enumerating them consecutively in a dorsal direction from the hilum) crossed retinal fibres end in laminae 1, 4 and 6, while uncrossed fibres end in laminae 2, 3 and 5. Moreover, it has been pointed out (Clark, 1941) that from each discrete spot in each retina fibres pass back to terminate in all three of the corresponding cell laminae in the lateral geniculate body, and it is inferred, therefore, that the conducting unit in the optic tract is a three-fibre unit. This laminar organization of the lateral geniculate body (consisting of two sets of three laminae) seems to be a feature common to all the catarrhine primates. It is certainly present in *Macaca*, *Presbytis*, *Cercopithecus*, *Papio*, all the genera of the anthropomorphous apes (Pongidae) and in *Homo*. Preliminary observations on the brains of a marmoset and a capuchin monkey, however, showed that in these New World primates the laminar differentiation is less elaborate and much less regular. A study of the lateral geniculate body in the platyrrhine monkeys generally was therefore undertaken.

The material for this investigation was provided by a series of brains which had been collected during past years from the Zoological Society of London. A block containing the termination of the optic tract and the lateral geniculate body was removed in each case and embedded in paraffin. Serial sections (15 μ) were prepared, being cut transversely to the axis of the optic tract. This orientation is necessary to allow of the accurate comparison of the somewhat complicated organization of the geniculate body in the different species. The sections were stained with toluidin blue. Since the material had undergone a prolonged immersion in formalin, the staining was not sufficiently favourable to permit of reproduction by photomicrography. A scale drawing was therefore made of each specimen at the transverse level where the laminar organization was most conspicuously developed (which proved to be at a level somewhat posterior to the middle of the nucleus), and the arrangement of the cells was

represented by free-hand drawing from direct observation of the section under a low-power objective. The accompanying illustrations all show transverse sections of the geniculate body at a common magnification of 20 diameters, and in all cases the medial aspect is to the left. It is believed that these drawings provide a sufficiently accurate representation of the cytoarchitectural appearance of the cell laminae. In the following descriptions the weights of the brain are given to the nearest gram. Measurements for the geniculate bodies are also recorded. The antero-posterior diameter has reference to the axis of the optic tract and was estimated by counting the sections in which the nucleus appears; the maximal oblique width was taken from the medial to the lateral margin of the nucleus, that is, along an oblique axis directed ventro-laterally, and in some cases, almost vertically, to the base of the brain. Lastly, the maximal oblique height represents the over-all diameter across the cell laminae measured perpendicularly to their axis as seen in transverse section.

The only previous observations on the lateral geniculate body of platyrrhine monkeys of which we are aware are descriptions of the nucleus in *Cebus* by Balado & Franke (1937) and in *Hapale* by Woollard (1926). In the latter account, however, attention was not given to the laminar pattern.

DESCRIPTIVE OBSERVATIONS

Marmoset (*Hapale jacchus*; see Fig. 1). Brain weight 8 g. The dimensions of the lateral geniculate body in this specimen are as follows: antero-posterior 3·9 mm., maximal oblique width 3·4 mm., maximal oblique height 1·9 mm. Throughout the extent of the nucleus the cell laminae are disposed almost vertically, but with a slight inclination dorso-medially. They are only moderately curved, so as to produce a shallow hilum directed medially. Towards the caudal end of the nucleus the hilum becomes more acute. At the anterior end one large- and one small-celled lamina are evident. As the sections are traced caudally the large-celled element rapidly splits into two laminae which are separated by a clear interval throughout most of their antero-posterior extent. They tend to fuse dorsally, however, towards the caudal end of the nucleus. In all sections the superficial large-celled lamina is somewhat wider than the deeper layer. The small-celled element forms a single mass rostrally but, when the second quarter of the geniculate body is reached in tracing the sections backwards, two separate laminae become evident in its ventral half. These small-celled laminae are of approximately equal width, and they are fused together in the dorsal half of the nucleus. A little farther back the whole small-celled element shows a definite tendency to become divided into two laminae, but the latter are at no transverse level completely separated in the dorsal half from each other by an uninterrupted acellular zone. In the posterior third of the geniculate body, the small-celled element again appears as a single mass of cells, with no laminar differentiation. At the caudal extremity, the large-celled laminae disappear, and at this level the ventral portion of the small-celled element becomes considerably thickened. Finally, between the large-

and small-celled elements are scattered lightly staining neurones of very small size, but these do not become aggregated to form a recognizable layer.

To summarize, the lateral geniculate body of the marmoset consists throughout the greater part of its extent of two well-defined large-celled laminae, and of two small-celled laminae which, though distinct in the ventral half of the nucleus through many sections, are otherwise not completely separated from each other.

Squirrel monkey (*Saimiri sciurea*; see Fig. 2). Brain weight 21 g. The dimensions of the lateral geniculate body are as follows: antero-posterior 6·0 mm., maximal oblique width 2·8 mm., maximal oblique height 1·6 mm. It will be noted that in this species the nucleus is more than twice as long as it is wide. At the site of entrance of the optic tract, the cell laminae are disposed horizontally. In the caudal half of the nucleus, however, their lateral margins turn ventrally, so as to produce a definite hilum which is directed medio-ventrally. In the rostral sections a thin large-celled and a thick small-celled

Fig. 1. Transverse section through the lateral geniculate body of *Hapale jacchus*. ×20.

Fig. 2. Transverse section through the lateral geniculate body of *Saimiri sciurea*. ×20.

lamina are evident. Traced caudally the large-celled element soon divides into two separate laminae, of which the more superficial is the larger throughout its extent. These laminae are everywhere clearly separated from each other by an acellular zone. Although the small-celled element is relatively thick, and although here and there in the transverse sections its constituent cells show a tendency to be arranged in somewhat ill-defined bands, it is in no region of the nucleus clearly laminated. Extremely small and lightly staining nerve cells are present in close relation to the large-celled laminae and form irregular layers of which one is placed superficially at the hilum while the other is interposed between the deeper large-celled laminae and the small-celled element.

It will be noticed that the lateral geniculate body of the squirrel monkey is very similar to that of the marmoset, except that it is more elongated in its general shape, and that the small-celled element is not clearly laminated.

Three-banded Douroucouli (*Aotes trivirgatus*; see Fig. 3). Brain weight 17 g. The dimensions of the lateral geniculate body are as follows: antero-posterior 2·4 mm., maximal oblique width 3·0 mm., maximal oblique height 1·2 mm. The cell laminae are disposed obliquely in a ventro-lateral direction; posteriorly they become practically vertical. The whole nucleus appears very flattened, but at most levels, as seen in transverse sections, the laminae are slightly curved so as to produce a shallow hilum directly medio-ventrally. In the most rostral sections are seen one layer of small cells and one layer of large cells; these rapidly divide as the sections are followed caudally, so as to form two small-celled laminae above and two large-celled laminae below. At the junction of the anterior and middle thirds of the nucleus the small-celled laminae become blended at their dorsal margins, and a few sections farther back the cytoarchitecture of the dorsal third of the small-celled element becomes confused and very irregular. In some sections, however, there is a grouping of the cells in this region which faintly indicates a subdivision into three or four rudimentary laminae (see Fig. 3). But the arrangement of the cells actually varies rapidly from one section to another, so that it is not possible to recognize any definite or consistent laminar pattern here. The two main small-celled laminae, however, are always distinct and clear-cut in the ventral two-thirds of the geniculate body (although they tend to unite at their ventral margins), and at the caudal end of the nucleus the whole transverse extent of the small-celled element is divided into these two layers. The two large-celled laminae are well defined and separated from each other by a comparatively wide interval throughout the antero-posterior extent of the nucleus. In most sections the more superficial layer is slightly the thicker. In the caudal half of the geniculate body (except at the posterior extremity) numerous very small and pale-staining cells are collected into two thin and rather distinct laminae, one of which lies between the two large-celled laminae and the other in the interval separating the latter from the small-celled element.

Fig. 3. Transverse section through the lateral geniculate body of *Aotes trivirgatus*. ×20.

Spider monkey (*Ateles ater*; see Fig. 4). Brain weight 107 g. The dimensions of the lateral geniculate body are as follows: antero-posterior 6·7 mm., maximal oblique width 5·7 mm., maximal oblique height 2·5 mm. The optic tract in this monkey is exceptionally broad and flat, and related to its strap-like shape the geniculate body is correspondingly broad and flat when seen in transverse sections. Rostrally the nucleus consists of two almost horizontal laminae, one of small and one of large cells. The large-celled lamina quickly divides into two laminae, of which the more superficial is distinctly the thicker. These laminae are separated from each other and from the small-celled element by relatively

wide acellular zones. In the latter are to be found scattered neurones of very small size, but they do not form a definite layer. The small-celled element maintains a relatively homogeneous cytoarchitecture throughout its extent. Here and there, in odd sections, there is a slight indication of a tendency for the cells to become irregularly disposed in a laminated fashion, but such arrangements are not consistently maintained from one section to another, and they seem therefore to have no morphological significance. Towards the caudal end of the geniculate body the lateral extremities of all the laminae become turned ventrally; a shallow hilum is thus formed, directed ventro-medially.

It will be observed that, in spite of its somewhat large size, the geniculate body of the spider monkey has a relatively simple structure. In the absence of a definite laminar pattern in the small-celled element, it resembles that of the squirrel monkey. The contrast between the laminar differentiation of the large-celled element and the absence of lamination in the small-celled element is noteworthy.

Fig. 4. Transverse section through the lateral geniculate body of *Ateles ater*. ×20.

Woolly monkey (*Lagothrix humboldtii*; see Fig. 5). Brain weight 68 g. The dimensions of the lateral geniculate body are as follows: antero-posterior 3·9 mm., maximal oblique width 4·3 mm., maximal oblique height 2·2 mm. The cell laminae are disposed almost vertically, with a slight inclination ventro-laterally. They are moderately curved so as to produce a shallow hilum directed medially. Just behind the entrance of the optic tract, the large- and small-celled elements of the nucleus are apparent. Traced caudally, the former becomes quickly split into two well-defined laminae. Of these the more superficial is considerably the wider, suggesting a corresponding preponderance of crossed retinal fibres among those which are related terminally to these laminae. Behind the middle of the nucleus, the superficial large-celled layer becomes complicated by a splitting and bending, and in several sections it appears to be

divided into two subsidiary laminae. The deeper large-celled layer, on the contrary, retains its simple band-like appearance throughout its antero-posterior extent. The small-celled element shows an irregular cytoarchitecture in the rostral third of the nucleus. In the middle third, however, some degree of laminar differentiation becomes evident. Here, in its ventro-lateral portion, the cells are disposed in two quite well-defined laminae which fuse with each other dorsally. A few sections farther caudally three laminae are recognizable in the ventro-lateral third of the nucleus. Still farther back (towards the caudal end) a fourth layer of small cells appears for a few sections at the ventral pole, and at this level all the small-celled laminae can be followed up into the dorso-medial part of the nucleus. However, they are not everywhere separated from each other as seen in the transverse sections, being at many levels united at

Fig. 5. Transverse section through the lateral geniculate body of *Lagothrix humboldtii*. × 20.

Fig. 6. Transverse section through the lateral geniculate body of *Cebus fatuellus*. × 20.

their dorsal and ventral margins, and also irregularly fused with each other in the centre of the nucleus. Thus the laminar pattern, though clearly present, is somewhat obscure. It may be observed, further, that the deeper large-celled lamina is not everywhere clearly separated from the small-celled element, the cells of both intermingling freely at the ventral margin of the lateral geniculate body.

Brown capuchin (*Cebus fatuellus*; see Fig. 6). Brain weight 53 g. The dimensions of the lateral geniculate body are as follows: antero-posterior 6·5 mm., maximal oblique width 3·1 mm., maximal oblique height 1·9 mm. The cell laminae are evenly curved throughout the antero-posterior extent of the lateral geniculate body, producing a conspicuous hilum directed ventrally

and slightly medially. At the caudal end the hilum becomes shallower. In rostral sections the nucleus consists of two large-celled laminae ventrally, and two small-celled laminae dorsally. The former are well defined and circumscribed, and continue almost entirely separated from each other to their posterior extremities. The superficial of these two laminae is the larger. The two small-celled laminae are clear-cut and distinct from each other at rostral levels. As the sections are traced back, however, they become blended together in the medial half of the nucleus. Farther caudally, this common mass of cells becomes split up first into three layers and then into four. These laminae, however, are by no means sharply defined, and they vary considerably in their distinctness from section to section. Still farther back, the small-celled laminae in the lateral half of the geniculate body also become split up into three or four irregular layers, of varying widths in different sections, and often interconnected with each other. These layers extend medially into continuity with those in the medial half of the nucleus, but (as shown in Fig. 6) in the junctional region the lamination is confused and uneven. At its caudal end the medial half of the geniculate body presents a still more complicated appearance, for each of the main laminae here shows a tendency to split into two subsidiary laminae. The latter, however, are somewhat variable in their contour and width in different sections, and their outlines are by no means always clear-cut. Between the large- and small-celled elements there are scattered neurones of minute size, but they can hardly be said to form a definite cell lamina.

DISCUSSION

Of all the platyrrhine genera which have been studied, *Cebus* has a lateral geniculate body which most closely approaches the catarrhine primates in its cytoarchitectural features. The laminae are strongly arched, leading to the formation of a pronounced ventral hilum. Moreover, at some transverse levels six laminae are clearly recognized, two consisting of large cells and four of small cells. However, the laminar organization of the small-celled element is not consistently maintained throughout any considerable extent of the nucleus, and the laminae are in many places irregular and not sharply outlined. Frequently, also, they are interconnected in a diffuse manner so as to obliterate locally any basic pattern which may exist elsewhere. Thus the lateral geniculate body of *Cebus* is quite definitely less completely differentiated in comparison with the nucleus of the Old World monkeys. On the other hand, it is clearly more highly organized than the nuclei of the other New World monkeys which have been examined.

In *Lagothrix* the geniculate body shows a laminar organization of the small-celled element, but less definite than in *Cebus*. In one part of the nucleus, four small-celled layers can be recognized, but these are only locally differentiated. Elsewhere the small-celled laminae are irregular and diffuse. A feature of the lateral geniculate body in this specimen is the somewhat complex folding and

subdivision of the superficial large-celled lamina, that is, the layer which presumably receives crossed retinal fibres. In general, the laminae are by no means so sharply curved as they are in *Cebus*.

The lamination of the geniculate body in *Aotes* is of a simple type. The whole nucleus is very flattened, so that the hilum is extremely shallow. The small-celled element is divided throughout most of its extent into two main laminae, though there is a faint indication of a further subdivision in its dorsal part in certain sections. Yet the laminar differentiation might have been expected to be much more rudimentary, for this genus (sometimes called the 'night ape') is nocturnal in its habits and it possesses a retina of the nocturnal type. Indeed, according to Woollard (1927) the retina is not only devoid of cones but also lacks a fovea and macula, and in these respects it is apparently unique among the Pithecoidea. Yet the geniculate body is as highly organized in its cyto-architectural differentiation as that of *Hapale* in which (also according to Woollard) the retina contains abundant cones as well as a macula. In fact, the nucleus is somewhat more highly differentiated in *Aotes*, for the minute and pale-staining nerve cells in relation to the large-celled laminae are themselves aggregated into two well-defined layers. The significance of these small and inconspicuous neurones is not known. They are to be found in the geniculate body of *Macaca*, and in this species they often form quite a well-defined layer below (superficial to) the large-celled laminae, though the definition of the layer appears to vary from one individual to another. It remains possible, of course, that the nocturnal type of retina in the douroucouli is the result of a secondary regression from a diurnal type furnished with cones and with a macula, and that the degree of laminar organization in the geniculate body related to such a retina has persisted without a corresponding change. Such a speculation, however, is based on no concrete evidence. It may be noted that the total size of the geniculate body in this monkey is very small in relation to the brain weight.

In *Ateles*, in relation to the large brain, the geniculate body is of a considerably greater size. Its laminar organization, however, is quite elementary, for the small-celled element nowhere shows any subdivision to form separate and well-defined laminae. Moreover, the whole nucleus is flattened, and the ventral hilum which is so characteristic of the geniculate body in catarrhine monkeys is but feebly developed. On the other hand, as in the other specimens, the large-celled element is conspicuously divided throughout the extent of the nucleus into two quite separate laminae. The lateral geniculate bodies in *Hapale* and *Saimiri* are very similar to each other, but in the former the small-celled element is in general bilaminar.

The significance of the differences in the laminar organization of the lateral geniculate body in the platyrrhine monkeys is obscure. It is evidently not related to the size of the brain as a whole, or to the total size of the nucleus, for it is least complicated in *Ateles*, and it is more differentiated in *Hapale* than in *Saimiri*. Moreover, it is much more highly developed in catarrhine monkeys

than in platyrrhine monkeys of comparable size. Reference has already been made in the introduction to the evidence that the conducting unit in the optic tract of the macaque monkey is a three-fibre unit, and this observation has naturally given rise to a consideration of the possibility that this may be related to the trichromatic theory of colour vision (Clark, 1941). It is difficult to investigate the existence of such a relation by direct methods, but at the commencement of this study it was hoped that comparative observations on the laminar organization of the geniculate body in platyrrhine monkeys might throw further light on the problem. In a recent review of colour vision in monkeys, Grether (1939) has brought forward evidence to show that *Cebus* possesses dichromatic vision, and it seemed possible that this might be related morphologically to the lower grade of laminar differentiation in the geniculate body of this genus as compared with the condition in catarrhine primates. However, the same author found that in a spider monkey colour discrimination was fully equal to that of the Old World monkeys, and we have seen that in this species the laminar organization of the geniculate body is even simpler than it is in *Cebus*. It is possible that a 'concealed' lamination of the small-celled element actually does exist in *Ateles*, in the sense that crossed and uncrossed retinal fibres terminate in different alternating zones of the element (as in *Macaca*), even though these are not visibly manifest in the cytoarchitecture of normal preparations. Such an intrinsic organization could only be determined experimentally by the study of transneuronal degeneration following section of one optic nerve. In this connexion, it may be noted that the *visible* lamination of the geniculate body in the Old World primates is brought about, not by the arrangement of the entering optic fibres, but by the intervention of medullary laminae composed of the geniculo-cortical fibres (Glees & Clark, 1941). Consequently, the absence of visible lamination in the small-celled element of the geniculate body in some of the New World monkeys may be related to the organization of the geniculo-cortical system rather than to the nature of the retino-geniculate connexions.

One outstanding feature which is emphasized by this study is that, however variable the lamination of the small-celled element of the lateral geniculate body may be in the platyrrhine monkeys, the large-celled element is consistently differentiated to form two clear-cut and distinctly separate laminae (presumably receiving crossed and uncrossed retinal fibres respectively). This seems, therefore, to be a fundamental character of the lateral geniculate body in all the Anthropoidea, and may be deemed to be related to some type of visual mechanism which is distinctive of this group.

From the phylogenetic point of view, it may be noted that the lower grade of laminar organization in the New World primates as contrasted with the Old World primates is consistent with the view, based on other morphological evidence, that the former occupy a lower status in the evolutionary scale.

SUMMARY

1. A description is given of the laminar organization of the lateral geniculate body in six genera of New World monkeys.

2. The laminar organization is less highly differentiated in these monkeys than it is in the catarrhine primates.

3. Within the group of New World monkeys the laminar organization is most highly differentiated in *Cebus*, and least in *Ateles* and *Saimiri*.

4. The significance of the variability in laminar organization is discussed.

REFERENCES

Balado, M. & Franke, E. (1937). *Das Corpus Geniculatum Externum.* Berlin.

Clark, W. E. Le Gros (1941). *J. Anat., Lond.*, **75**, 225.

Glees, P. & Clark, W. E. Le Gros (1941). *J. Anat., Lond.*, **75**, 295.

Grether, W. F. (1939). *Comp. Psychol. Monogr.* **15**, 1.

Woollard, H. H. (1926). *Brain*, **49**, 77.

—— (1927). *Proc. Zool. Soc., Lond.*, April, p. 1.

REVIEW

A Bibliography of Human Morphology, 1914–1939. By W. M. KROGMAN. Cambridge University Press. Pp. xv+385. Price 18*s*.

Dr Krogman has performed a very useful service in compiling this bibliography. As stated in the preface, it was intended to follow on the first edition of Martin's *Lehrbuch der Anthropologie* by providing references to the works on physical anthropology which had been published since then. Actually, however, it covers a considerably broader field. We find here and there some seemingly incongruous references —e.g. under the heading of Viscera, a paper on the weight of ductless glands in the Norway and Albino Rat; under the heading of Nervous System, papers on the morphology of the corpus striatum and the cerebrospinal fluid and lymph nodes; and a section on mammals generally with such references as 'The diving power of whales', 'The giraffe and his living ancestor', and 'The structure of the skull in a Dinocephalian reptile'. It is not easy to understand why such heterogeneous references should be included in a bibliography intended for the use of the student of physical anthropology. Nevertheless, we repeat that the author has performed a useful service by this compilation. For the information of readers, the reviewer estimates that the bibliography contains somewhat more than 10,000 references.